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VOL. 54 FEBRUARY, 1961 NO. 1

CONTENTS

Sepedon macropus (Diptera: Sciomyzidae) Introduced into Hawaii as a Control for the Liver Fluke Snail, *Lymnaea ollula*. . . . Q. C. Chock, C. J. Davis and M. Chong 1
Artificial Media and Rearing Techniques for the Pink Bollworm. . . . Edgar W. Clark, Clyde A. Richmond and James M. McGough 4
The Metabolism of C^{14}-Labeled DDT in the Larvae, Pupae, and Adults of *Drosophila melanogaster*. . . . Daniel B. Menzel, S. M. Smith, Raymond Miskus and W. M. Hoskins 9
Laboratory and Field Studies on Orchard-Mite Resistance to Kelthane. . . . Stanley C. Hoyt and Ford H. Harries 12
Sorghum as a Host of the Southwestern Corn Borer. . . . James R. Gifford, R. R. Walton and K. D. Arb[illegible]thnot 16
Germination of Small Legume Seeds After Fumigation with Methyl Bromide and Hydrocya[illegible]c Acid. . . . R. G. Strong and D. L. L[illegible]dgren 21
Residues in Tissues and Eggs of Poultry Dusted with Co-Ral (Bayer 21/199). . . . H. W. Dorough, U. E. Brady, Jr., J. A. Timmerman, Jr. and B. W. Arthur 25
The Role of Attractants in the Recent Mediterranean Fruit Fly Eradication Program in Florida. . . . L. F. Steiner, G. G. Rohwer, Ed L. Ayers and L. D. Christenson 30
Control of the Tadpole Shrimp, *Triops longicaudatus*, in California Rice Fields. . . . A. A. Grigarick, W. H. Lange and D. C. Finfrock 36
Preliminary Study of the Genetics of House Fly (*Musca domestica*) Resistance to Malathion. . . . Robert L. Harris, Stanley Wearden and Clifford C. Roan 40
Effect of Some Variations in Formulation or Application Procedure on Control of the Imported Fire Ant with Granular Heptachlor. . . . C. S. Lofgren, V. E. Adler and W. F. Barthel 45
Control of the Cabbage Looper with a Nuclear Polyhedrosis Virus Disease. . . . John C. Elmore 47
Further Studies of Integrated Control Methods against the Egyptian Alfalfa Weevil in California. . . . Vernon M. Stern 50
Factors Influencing Citrus Red Mite Populations on Navel Oranges and Scheduling of Acaricide Applications in Southern California. . . . L. R. Jeppson, J. O. Complin and M. J. Jesser 55
Control of Spider Mites and Black Spot on Roses with Acaricide-Fungicide Sprays and Dusts Thomas J. Henneberry, Edgar A. Taylor, Floyd F. Smith, A. L. Boswell and W. D. McClellan 61
Hercules AC-5727 as a Residual Spray for Adult Mosquitoes. . . . J. B. Gahan, G. C. LaBrecque and H. G. Wilson 63
The Cotton Leaf Perforator and Its Control in Arizona. . . . Donald M. Tuttle, George P. Wene and Louis W. Sheets 67
Field Studies with Baits Against *Solenopsis saevissima* v. *richteri*, the Imported Fire Ant. . . . F. J. Bartlett and C. S. Lofgren 70
An Evaluation of Coding Systems for Estimating Populations of the Shaft Louse, *Menopon gallinae*. . . . J. C. Harshbarger and E. M. Raffensperger 74
Effect of Migration, Temperature, and Precipitation on Thrips Infestations in South Texas. . . . James A. Harding 77
Comparison of Ronnel, DOWCO 109, and DOWCO 105 for Systemic Control of Cattle Grubs in Alberta. . . . J. Weintraub and C. O. M. Thompson 79
Artificial Infestations of the Northern Cattle Grub, *Hypoderma bovis*, in Texas. . . . J. Weintraub, W. S. McGregor and H. M. Brundrett 84
Toxicity of TDE to Different Instars and Strains of the Red-Banded Leaf Roller and the Response of a TDE-Resistant Strain to Parathion and Endrin. G. C. Rock, C. H. Hill and J. M. Grayson 88
The Ticks (Argasidae and Ixodidae) of Turkey. . . . Dale W. Parrish 91
Studies of an Integrated Control System for Hornworms on Tobacco. . . . F. R. Lawson, R. L. Rabb, F. E. Guthrie and T. G. Bowery 93
Residues in Tissues and Eggs of Poultry Receiving Co-Ral (Bayer 21/199) in the Feed. . . . H. W. Dorough, U. E. Brady, Jr., J. A. Timmerman and B. W. Arthur 97
Influence of Caging and Transferring Techniques on Aphid Mortality and Virus Transmission. . . . Paul P. Cook and Edward S. Sylvester 101
Inhibition of House Fly Ali-Esterase and Cholinesterase Under *in vivo* Conditions by Parathion and Malathion. . . . Frederick W. Plapp, Jr. and Walter S. Bigley 103
An Ecological Approach to the Insecticide Problem. . . . David Pimentel 108
Field Studies on the Control of Resistant House Flies. . . . H. B. Weinburgh, J. W. Kilpatrick and H. F. Schoof 114

Lygus Bug Damage to Table Beet Seed Plants ... Elmer C. Carlson 117
Insecticidal Susceptibility of *Nysius raphanus*, a Pest of Cotton ... Thomas F. Leigh 120
Effect of Certain Antibiotics and Other Compounds on the Two-Spotted Spider Mite ... F. H. Harries 122
Incidence of Field Spread of Internal Cork of Sweet Potato in Insecticide-Treated Plots ... E. J. Kantack, W. J. Martin and L. D. Newsom 125
Hazards Associated with the Implantation of Tetram into Elm Trees for Dutch Elm Disease Control ... Abdulla F. Al-Azawi, Dale M. Norris, Jr. and John E. Casida 127
Susceptibility of an Eye Gnat, *Hippelates pusio*, to Residual Insecticides ... Andrew Spielman 130
The Development and Characterization of Resistance to Carbamate Insecticides in the House Fly, *Musca domestica* ... G. P. Georghiou, R. L. Metcalf and R. B. March 132
Evaluation of Chemical and Microbial Materials for Control of the Cabbage Looper ... I. M. Hall, R. L. Hale, H. H. Shorey and K. Y. Arakawa 141
Effects of Insecticides on the Predaceous Arthropod Fauna of Louisiana Sugarcane Fields ... S. D. Hensley, W. H. Long, L. R. Roddy, W. J. McCormick and E. J. Concienne 146
Synergism of Carbamate Insecticides with Octachlorodipropyl Ether ... G. P. Georghiou and R. L. Metcalf 150
Control of European Brown Snail in Citrus Groves in Southern California with Guthion and Metaldehyde Sprays ... Joseph L. Pappas and Glenn E. Carman 152
An Evaluation of Insect Resistance in Broccoli, Brussels Sprouts, Cabbage, Collards, and Kale ... David Pimentel 156
Translocation and Stability of Chipman R-6199 in *Pinus strobus* as Related to Its Control of the Introduced Pine Sawfly, *Diprion similis* ... Dale M. Norris, Jr. and Harry C. Coppel 159
Control of Pea Enation Mosaic in Peas with Insecticides ... A. C. Davis, F. L. McEwen and W. T. Schroeder 161
A Laboratory Evaluation of Ethion with Other Acaricides Against the Adult Two-Spotted Spider Mite, *Tetranychus telarius* ... Paul C. Lippold 166
The Effect of Tedion on the Eggs and Larvae of Three Strains of the Two-Spotted Spider Mite, *Tetranychus telarius* ... T. J. Henneberry, E. A. Taylor and A. L. Boswell 168
Laboratory and Field Tests of New Insecticides for Grasshopper Control ... F. E. Skoog, F. T. Cowan and R. V. Connin 170
Loss of Parathion and DDT to Soil from Aqueous Dispersions and Vermiculite Granules ... Donald E. Weidhaas, Malcolm C. Bowman and Claude H. Schmidt 175
Insecticidal Control of the Sugar-Beet Root Maggot and Yield of Sugar Beets ... W. R. Allen, W. L. Askew and K. Schreiber 178
Effect of Insecticide Fertilizer Mixtures and Seed Treatments on Emergence of Sugar Beet Seedlings ... W. R. Allen, W. L. Askew and K. Schreiber 181
The Uptake and Metabolism of Parathion by Insect Eggs ... R. D. O'Brien and E. H. Smith 187

Scientific Notes

Emergence of Overwintered Pink Bollworm in Arizona ... George P. Wene, L. W. Sheets and H. E. Woodruff 192
Control of the Beet Armyworm on Cotton in Arizona ... George P. Wene and L. W. Sheets 192
Scabbing of Rutabagas After Applications of Heptachlor, Parathion, and Lime to Mineral Soils of Various Acidities ... D. C. Read and D. B. Robinson 193
Influence of Water and Previous Food on the Longevity of Unfed Larvae of *Coleomegilla maculata lengi* ... Bernard C. Smith 194
Biological Notes on Five Recently Imported Parasites of the Egyptian Alfalfa Weevil, *Hypera brunneipennis* ... T. W. Fisher, E. I. Schlinger and R. van den Bosch 196
Control of Millipedes in Greenhouse Soil ... T. J. Henneberry and E. A. Taylor 197
Metabolism of Injected Proline in DDT-Poisoned German Cockroaches ... Walter Hoy and H. T. Gordon 198
Aerosol Dispensing System for Aircraft Disinsectization ... A. H. Yeomans, R. A. Fulton and W. N. Sullivan 199
Evaluation of Insecticides for Control of the Tobacco Thrips on Peanuts ... James A. Harding 200
Effect of Gamma Radiation on the Reproductive Potential of the Mexican Fruit Fly ... R. H. Rhode, F. Lopez D., F. Eguisa and J. Tellich C. 202
Field Tests with New Cattle Grub Systemics ... A. R. Roth and Gaines W. Eddy 203
Effectiveness of Insecticides against the Rusty Plum Aphid and Ants ... Leon J. Charpentier 204
Effect of Captan upon Reproduction in the Two-Spotted Spider Mite, *Tetranychus telarius* ... Preston E. Hunter 204
Occurrence of *Hippelates* Eye Gnats in a Coastal Habitat in California ... Harvey I. Magy and Arthur A. Lee 206
Parasitization of *Coctoblastis cactorum* by *Trichogramma semifumatum* in Hawaii ... Alden D. Hinckley 207
Control of Cattle Grubs and Horn Flies by Summer Dipping with Co-Ral ... Joseph S. Simco and J. L. Lancaster, Jr. 208
Residues of Phosdrin® on Alfalfa and Its Effectiveness on the Insect Complex ... E. W. Huddleston and George G. Gyrisco 209
An Electrically Heated Retaining Pen for Insect Larvae J. P. Hollingsworth, C. P. Briggs III and A. L. Scales 210
The Effects of Gamma-Ray Irradiation on Certain Species of Stored-Product Insects ... Norman M. Dennis 211
Population Fluctuations of *Lydella grisescens*, A Parasite of the European Corn Borer ... J. L. Jarvis and G. T. York 213
New Price Schedules ... 214
Book Reviews ... 116, 215
Announcements ... 60, 92, 116, 169, 216
Obituary: Herbert Dwight Smith (1894–1959) ... 217

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Vol. 54 February, 1961 No. 1

Sepedon macropus (Diptera: Sciomyzidae) Introduced into Hawaii as a Control for the Liver Fluke Snail, *Lymnaea ollula*[1]

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Abstract

Liver fluke, *Fasciola gigantica* Cobbold, is the most important parasitic disease of beef and dairy cattle in the State of Hawaii and has been in the Islands since 1892. The intermediate host of this parasite is the fresh water snail, *Lymnaea* (Fossaria) *ollula* Gould. Another aquatic snail, *Pseudosuccinea columella* Say has proven to be a vector of liver fluke under laboratory conditions. Both snails are found in streams, taro paddies and swamp habitats. Because of the importance of this problem, *Sepedon macropus* Walker was introduced in an attempt to control these aquatic snails.

Under Hawaiian climatic conditions, the life cycle of this predator is approximately 22 to 24 days. The egg stage is 3 days, larval stage 11 to 12 days and pupal stage 7 to 8 days. Two methods were employed in rearing the larvae and these consisted of the use of petri dishes and redwood troughs. The former was employed initially to increase the breeding stock, while the latter was applied later in order to obtain mass production.

Nutrition studies indicated that greater fecundity and longevity were obtained by a diet of crushed snails, water and honey, made available to the breeding stock. The males outlived the females; the average life span of the male was 100 days, that of the female was 69 days. The ratio of egg production as indicated in these studies was 184 eggs obtained in which the normal diet was honey and water as against 14,824 eggs, when crushed snails were added.

Releases of *S. macropus* have been made on the five major islands of the State, and to date recoveries of the fly have been reported only from Oahu and Kauai. In areas where establishment of the fly has occurred, usually half an hour of trapping will result in the capture of 50 to 100 flies, indicating the adaptability of this sciomyzid species to Hawaiian conditions. Judging by the encouraging progress taking place, it appears that this introduced predator may become an important contribution in the control of *Fasciola gigantica* in the State of Hawaii.

According to Alicata (1946) the most important parasitic disease of beef and dairy cattle in the Hawaiian Islands is caused by the liver fluke (*Fasciola gigantica*) Cobbold. Liver fluke infection here dates back at least to 1892, when it was first reported on Kauai, Oahu, Maui and Molokai. Animals slaughtered at that time revealed 298 calves out of 620 and 1,313 cattle out of 2,186 were infected with flukes. In 1931, the disease was considered widespread throughout the State. During 1958, a total of 24,141 cattle were slaughtered and infection was found in 6,633 cases. At current market prices, the loss of approximately 66,330 pounds of liver was estimated at $34,823.25. Losses attributable to the fluke from poor weight gains, decreased milk production, death of animals, et cetera, amount to a far greater figure, which cannot be accurately measured. These data were obtained from the Division of Animal Industry of the Department of Agriculture and Conservation of the State of Hawaii, which keeps a record of all cattle slaughtered in the Islands (Willers 1958).

Intermediate Hosts of *Fasciola gigantica*.—In Hawaii, *Lymnaea ollula* Gould and *Pseudosuccinea columella* Say, commonly found in streams, taro and water cress patches are the carriers of the flukes; other snails present in similar areas have not been found to serve as hosts.

There are three important phases in the life cycle of *F. gigantica*: (1) development in the snail, (2) encystment on vegetation, and (3) development in the cattle. Although strict application of measures on any one stage would help control the parasite, it is in relation to phase (1) that an attempt through biological control is being made as an indirect means of controlling the parasite.

Early Efforts at Biological Control of Snail Pests.—Numerous attempts have been made by local institutions in previous years to introduce and colonize lampyrids for the control of *Lymnaea ollula* and *Achatina fulica* Bowdich. These lampyrids consisted of *Proturis hebes* Porter from the United States, *Colophotia praeusta* Esch. from the Philippines and *Luciola cruciata* Motsch. and *L. lateralis* Motsch. from Japan. Fairly large numbers of adult fireflies of *Colophotia* and *Luciola* spp. were obtained and released directly in the field. Unfortunately, none of the species liberated has been recovered. *Colophotia praeusta* was the only species that was successfully propagated in the insectary at the time. Rearing was done in an insectary building approximately 16′×30′×16′ with concrete floor, a combination 4′ tile wall on which glass was utilized to the remaining height and glass was used for the roof. Breeding trays 30″×6′×6″ on tables were set along the walls and a row in the center of the insectary. These trays were half filled with sterilized soil and a layer

[1] Accepted for publication June 13, 1960.

[2] State Entomologist, Assistant State Entomologist, and Insectary Supervisor, respectively.

of dampened sphagnum moss approximately 2 inches thick covered the surface sparsely. The breeding stock was then released and the fireflies soon alighted on the moss in the trays and deposited eggs. When eggs were observed, usually in large numbers, crushed Chinese snails (*Vivipara chinensis* Gray) were provided as food for the larvae. The Chinese snails were collected in taro (*Colocasia esculenta*) patches, where they were especially plentiful. Pupation took place in the trays and the adult fireflies emerged and were collected and released in the field. This operation continued for more than 6 months and thousands of fireflies were thus reared and liberated in the Islands.

Tests with Sciomyzidae.—As a result of a grant received by Dr. Clifford O. Berg, Department of Entomology and Limnology, Cornell University, Ithaca, New York from the U. S. Public Health Service to collect and study the Sciomyzidae, funds were allocated to Stuart E. Neff, to accompany Eric Mathews, who had received a grant to engage in a study of the behavior of the Scarabaeidae in Mexico and Central America. They traveled the Inter-American Highway from the United States to Mexico, Guatemala and Nicaragua, thus collecting was confined to a rather narrow strip. In the first few days, stops were made at every water hole along the highway for collecting. Since Dr. Berg had taken the closely related *Sepedon caerulea* Mel. 6 months earlier in Puerto Rico, Mr. Neff, therefore, was determined to capture *Sepedon macropus* on this trip. The first fly capture was a female and it was collected by sweeping over cat-tail (*Typha latifolia* L.), and an undetermined shrub growing in an area 30 miles southwest of Guatemala City and at an elevation of 4,500 feet. This was on July 21 and subsequently on July 27, 5 females and 7 males were collected 7 to 8 miles north of Managua, Nicaragua. The elevation of this area is 200 feet above sea level, flat with broad pastures. The flies were captured along the streams and in swamps, where *Typha* and other weeds made up the vegetation.

Dr. Berg's interesting studies on the snail-killing sciomyzid flies prompted us to make further attempts in the biological control of *Lymnaea ollula*, and possibly *P. columella* which, under laboratory conditions, were found to be alternate hosts of the liver fluke, *Fasciola gigantica*, by introducing marsh flies. Being a specialist in the Sciomyzidae, Dr. Berg had several species of marsh flies in his laboratory at the time our request reached him. He was anxious to have experiments conducted with the various sciomyzid species to determine their effectiveness in the control of *Lymnaea* snails in the State of Hawaii, because of the ecological conditions where biological control has been practiced with a high degree of success for many years and is continuing. Fortunately at the time, he had available a first generation *Sepedon macropus* larvae obtained in his laboratory from flies shipped to him 2 weeks previously by his collector. He made two shipments of larvae to us in various instars, which arrived on October 8 in excellent condition. There were 38 and 20 larvae recovered in the respective shipments. With detailed instructions on the techniques in the rearing of these larvae, a total of 25 flies were obtained, which made up our original breeding stock.

BREEDING TECHNIQUE.—The breeding stock consisting of 10 pairs of adults was held in a gallon-size fruit jar with a wide mouth. It was set horizontally and a 1″×2″ section of sponge saturated with water was placed about the middle of the jar at the bottom to provide water for the flies. A bouquet of Buffalo grass, *Stenotaphrum secundatum* (Walt.) Kuntze, set in a 1″×4″ vial filled with water, was then tilted on the sponge at an angle of 45 degrees. This was to prevent the escape of water from the vial and to allow maximum space for the movement of the flies. Honey and crushed *Lymnaea* snails were made available as food. A half teaspoonful of crushed snails was used in each jar.

Life Cycle.—The life cycle of *Sepedon macropus* is approximately 22 to 24 days, under Hawaiian conditions, as follows:

Duration of: Egg stage	3 days
Larvae	11 to 12 days
Puparia	7 to 8 days
Preoviposition period of females	4 days

Adults.—Preoviposition period of the newly emerged females was 5 days, when honey was provided, and 4 days with crushed snails added to the diet. The addition of crushed snails apparently caused the flies to become unusually active and sexually aroused. Copulation was soon observed and within a day or two oviposition took place. Under insectary conditions the male flies outlived the female flies. Studies made indicated that a total of eight females made final deposition of eggs and lived from 3 to 23 days thereafter, and the remaining 19 females died within 1 or 2 days following last oviposition.

Eggs.—Eggs were laid side by side in rafts or clutches anywhere from a few to as many as 38. They were fastened on the upper surface of grass blades and were also found attached to the sides of the jar. Newly laid eggs were white, but they gradually turned tan or brown. Infertile eggs remained white.

The grass blades with egg clutches were clipped daily and placed on dampened filter paper and held in 4″ petri dishes with covers. Egg clutches attached to the jar were not removed; instead, the jar was filled with water and held until eggs hatched. The eggs normally hatch in 3 days in the petri dishes and in the jar containing water.

Larvae.—Larvae were reared in petri dishes initially and food consisted of crushed snails, and young snails. They were also fed snail egg masses with well developed embryos when available. Fresh food was provided daily and as the larvae developed, larger snails were provided. For sanitation purposes, larvae were transferred to clean containers daily. This was accomplished by floating larvae to the surface by the addition of water and transferring the larvae with a camel's-hair brush.

The petri-dish method employed initially assured a nucleus of stock and in order to increase production substantially, redwood tanks or troughs measuring 47″×16¾″ ×15½″ were utilized. These were divided into two compartments (A and B) and stocked with aquatic plants such as water hyacinth (*Eichormia crassipes* Solms, *Ludwigia palustris* Ell. and *Monochoria vaginalis* (L.)). Water hyacinths were particularly attractive to *P. compacta* for egg-mass deposition. When snail egg masses were abundant, 500 to 1,000 first or second instar larvae were placed in compartment A at water inlet and held

until the third instar. At this time the baffle was removed and the larvae were allowed to enter compartment B, which was well stocked with all stages of *Lymnaea* and *Physa* snails.

Puparia.—Pupation took place on the sides of the trough at water level, in corners and among aquatic vegetation. Newly formed puparia appeared light tan ventrally and brown dorsally. They gradually turned dark brown and sometimes even black prior to the emergence of the flies. The puparia were collected from the petri dishes and troughs and placed on water-saturated sphagnum moss in 4″ petri dishes. To recover the puparia from the troughs, the aquatic plants were pressed below the surface of the water, thus exposing them by floating them to the surface. Each petri dish containing puparia was placed in a gallon fruit jar having the opening covered with a piece of cloth to await the emergence of adults. Pupal duration was 6 to 7 days with less than 2% mortality.

Prior to the use of the redwood troughs, the average number of flies produced per month for the first 3 months was 407. When they were activated in April, the total emergence increased to 1,537 and reached a peak of 4,500 in the month of May.

Predators.—In activating the redwood troughs, it soon became obvious that predators exacted a heavy toll on both snail and *Sepedon* populations. Both *Lymnaea ollula* and *Physa compacta* were preyed upon by the freshwater leech, *Glossiphonia weberi* Blanchard and, under laboratory conditions snails of all sizes were attacked. Small snails were preferred. Chernin *et al.* (1956) summarized their studies as follows: "The growth of an *A. glabratus* population was effectively controlled by the leech, *H. fusca*, under laboratory conditions. Small snails were found to be exceptionally vulnerable although snails of all sizes were killed by the leech. Furthermore, while the mature leeches were primarily responsible for killing the snails, it was found that young leeches may also play a contributory role."

Although every effort was made to exclude leeches from the troughs a few managed to enter undetected on the aquatic vegetation and snails, which were placed in the troughs. This nucleus population and resulting progenies seriously hampered the build up of natural snail populations and was an active competitor of *S. macropus* for food.

Brachydeutra hebes Cresson was another predator of the liver fluke snail under laboratory conditions, but was relatively unimportant.

For *S. macropus* the most important predators were dragonfly and damselfly naiads, which were brought in on aquatic plants and snails, *Pheidole megacephala* Fabr., which robbed the troughs of larvae and puparia and the hemipterous bug, *Mesovelia mulsanti*, which accompanied aquatic plants and preyed heavily on first instar larvae.

DISTRIBUTION.—Prior to the release of *Sepedon* in the field, a survey was conducted to locate areas where *Lymnaea* snails were plentiful. Such areas were in swamps, taro and water cress patches and along streams and drainage ditches. Since large areas in the State were planted in taro, and high incidence of *Lymnaea* snail populations occurred, the initial release and recovery of marsh flies reared locally was made on the major Islands as follows:

Initial Releases				Total Released to December 31, 1959
Island	Date	No. Released	Locality	
Oahu	December 15, 1958	8	Kahaluu	3,738
Hawaii	January 20, 1959	35	Honolua	1,280
Kauai	March 19, 1959	100	Hanapepe	1,684
Molokai	May 20, 1959	315	Keopukaloa	615
Maui	May 21, 1959	300	Lahaina	316
Total				7,633

Recovery				
Island	Date of Initial Release (1959)	Locality	Date (1959) Recovered	No. of Flies
Kauai	April 29	Waimea Ditch above Kekaha	June 10	2
Oahu	January 6	Waiahole	Sept. 23	33
Oahu	August 26	Waimalu	Dec. 15	12
Oahu	November 3	Luluku	Dec. 29	6

The total number of *Sepedon* trapped as of December 31, 1959 was 507.

The flies recovered on Kauai were captured by sweeping over vegetation along the Waimea ditch, while those on the island of Oahu were captured in traps, which were baited with crushed *Lymnaea* snails. Two types of traps were employed, the McPhail invaginated fruit fly (glass) trap and our home-made "double-barrel" trap. It is made of a cellophane cylinder 9″×12″ with openings at both ends. A funnel 9″ wide and tapering 5″ to an opening of $1\frac{1}{2}$″ is inserted at each end of the cylinder and taped securely. Each funnel is removable for the convenience in recovering the flies. This type of trap is set up horizontally in the field and approximately 2 feet off the ground, whereas, the McPhail trap is set up with the opening at the bottom. Both types of traps are efficient in capturing *S. macropus*. During a 4-day trapping at Waiahole, Oahu, in which approximately half an hour was spent each day, the results were as follows:

Date	No. of Flies
December 8, 1959	200
December 9, 1959	90
December 18, 1959	150
December 21, 1959	49
Total	489

FIRST LOCAL GENERATION.—There were 113 puparia obtained in the first local generation of *S. macropus* and these produced 105 flies consisting of 59 females and 46 males. Eight puparia failed to produce flies. Emergence was obtained in 6, 7, 8 and 9 days and the number of flies produced was 11, 32, 39 and 23, respectively. The

majority of the flies emerged between 7 and 8 days. Average temperature for the months of November and December in which the generation was reared was approximately 71° F. The sex ratio of the flies was 59 females and 46 males.

NUTRITION STUDIES.—Two series of studies for comparing the effect of diets consisting of honey and water; crushed snails and water; and crushed snails, honey and water were completed. Nine pairs of flies of *S. macropus* were utilized in each series.

Total eggs produced in both series when the flies were fed (1) honey and water was 184; (2) crushed snails and water 2,725; and (3) crushed snails, honey and water 14,824. In both series under these diets the males outlived the females; the greatest number of days lived by a male was 173 days and by a female 105 days. Average life in both series for the females was 69 days, and that for the males 105 days. The average life for the females and males was: female 48, male 50 days on diet 1; female 27, male 21 days on 2; and female 69, male 103 days on 3.

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Artificial Media and Rearing Techniques for the Pink Bollworm[1]

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ABSTRACT

Pink bollworms (*Pectinophora gossypiella* (Saund.)) were reared on two artificial media, consisting of cottonseed meal or peanut flour in combination with other constituents. A combination of antimycotic agents, n-butyl-*p*-hydroxybenzoate, sodium benzoate, and sorbic acid or potassium sorbate, when added to the media inhibited fungous growth for at least 10 days. The cannibalistic tendencies of the larvae were partially thwarted by furnishing a large area of medium per larva and/or segregating the larvae by subdividing the total mass of medium with or without physical barriers. Boll weevils and phorid flies were also reared on the cottonseed meal diet. Techniques for handling the eggs and larvae are described.

In many fields of entomological research a continuous, large supply of the insect under investigation is needed, and such is not always available from natural sources. Many studies require insect populations which are uniform in characteristics such as age and size. These requirements can be met by mass-rearing the insect under controlled conditions. Therefore, research was undertaken at the Brownsville, Tex., laboratory in 1954 to develop an artificial diet and make preliminary studies of a rearing technique for the pink bollworm, *Pectinophora gossypiella* (Saund.).

One of the major prerequisites of a mass-rearing technique is the development of an efficient dietary medium. Chemically defined media for the pink bollworm have been described by Beckman *et al.* (1953), Vanderzant (1956, 1957), Vanderzant & Reiser (1956), and Vanderzant *et al.* (1957). Vanderzant *et al.* (1956) also reported a rearing technique using cottonseed or peas and other legumes for a medium.

Inasmuch as a large amount of a chemically defined diet can be costly and difficult to prepare, a simple one made from commercially obtainable plant products was sought. The search was approached in two ways—by screening natural foods such as cottonseed, okra, and seeds of other host plants, and by using easily available derived products such as cottonseed meal.

METHODS AND MATERIALS.—Pink bollworm moths, needed for an egg supply, were obtained either from infested green cotton bolls or infested seed cotton, using a large Berlese funnel (Clark *et al.* 1959), emergence cages (Shiller 1946), or an emergence room (Richmond & Husman 1957). To obtain eggs, 20 to 200 moths were placed in an oviposition box which measured about 1 cubic foot. This box had a 5-inch hole at the top covered with 14-×18-mesh wire gauze and was coated inside with paraffin to discourage oviposition on the box itself. An aqueous fructose-glucose solution in a cotton-stoppered vial provided the food source for the moths. Washed cotton leaves, filter paper, or black paper was placed on the wire gauze to serve as an oviposition site. To remove the eggs, the site was soaked in a 50-p.p.m. aqueous solution of Tween 80 for 2 to 5 minutes, the eggs were brushed off into the solution with a camel's-hair brush, and this suspension was suction-filtered through Whatman No. 50 filter paper in a Buchner funnel. Just before all the solution was removed it was swirled with a stirring rod to create a vortex which concentrated the eggs in the center of the filter paper. The eggs were then either sterilized and stored for use in sterile rearing techniques developed by Stride (1953), or placed directly on the nutritional medium in attempts to develop nonaseptic techniques. To determine the effect of the sterilization technique on egg development, three replicates of about eighty 1-, 2-, 3-, and 4-day-old eggs were subjected to this procedure, and the eggs were observed for embryonic development and hatchability. No microbial contamination developed. In experiments involving the screening of potential anti-

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mycotic agents, the eggs were not always removed from the oviposition site.

Plant Materials.—Plant materials tried as a diet were: Fruit and flowers of two secondary host plants, *Pseudoabutilon lozani* Fries and *Malvaviscus drummondii* T. & G., sliced and mashed sweetpotatoes, okra seed and intact fresh okra pods of various ages, cottonseed, peanut flour, soybean flour, and cottonseed cake and meal. Dry and moist meals and cakes, and dry, moist, and sprouted seeds were used. The seeds were moistened by soaking them in water for 1 to 2 hours, after which they were thoroughly drained. The stems of the freshly cut pods were dipped in melted paraffin to retard fungous infection and desiccation of the pods, thereby extending their useful life several days.

Generally, plant materials were placed in 4.5- ×4.5- ×3-inch plastic refrigerator dishes, or 250-mm. Erlenmeyer flasks which were sealed with plastic covers or absorbent cotton plugs to retain the larvae and minimize fungous contamination. Pink bollworm eggs or first-instar larvae were introduced on the material. No aseptic conditions were maintained, but the equipment and glassware were thoroughly cleaned and, in some cases, autoclaved. In these and all succeeding experiments, the diets were evaluated by the following criteria: First, whether the diet supported larval growth and development; second, whether larval growth and developmental period were normal; and, third, what per cent of the introduced eggs became mature larvae or adults.

Two years after plant materials were screened at the Brownsville laboratory, Vanderzant *et al.* (1956) reported rearing pink bollworms using sprouted cottonseed. This technique was tried with subsequent modifications. Sound delinted Empire cottonseed were soaked for 4 to 8 hours in water, drained, spread on absorbent paper, and allowed to sprout in a darkened room at about 29° C. The sprouted seed were either sprayed with or immersed in one of the following three antimycotic solutions until wet: (1) 0.5 gram of Butoben (*n*-butyl-*p*-hydroxybenzoate) and 2 grams of methylcellulose (Dow Methocel 400 c.p.s.), as a dispersing and adhesive agent, per 100 ml. of water; (2) 2 grams of Butoben per 100 ml. of 25% ethanol; or (3) 0.22 gram of copper sulfate per 100 ml. of water. The seeds were then drained, air-dried, and placed on absorbent cotton in plastic 4.5- ×4.5- ×3-inch refrigerator dishes, 32-ounce canning jars, or shallow pans. Pink bollworm eggs were evenly distributed among the seed, and plastic covers or fine muslin was placed firmly on the containers. After the first-instar larvae had crawled into the seed mass and ceased wandering, the covers were removed to discourage mold growth and were not replaced until fourth-instar larvae matured. In addition to the preinfestation fungistatic treatment, experiments were conducted in which a second treatment was used after the seeds were infested. To prevent wandering of the first-instar larvae, a series of experiments were conducted in which mature eggs were placed on the bottoms of the containers or cylindrical battery jars; these were then covered with unbleached muslin. When the eggs began to hatch they were covered with a layer of the previously described sprouted seeds.

Commercial Plant Products.—Twenty-one artificial diets were tried using the following plant products as bases: Cottonseed meal, both with and without cottonseed oil; soybean meal; peanut flour; sesame seed, and ground carrot. Media were prepared by combining these products with other nutrients to produce a composition similar to that of a 20-day-old cotton boll (Bailey 1948, Guthrie *et al.* 1949). All media contained 70% to 75% of water since such a dietary content is required for development of nondiapause larvae (Bedford 1934, Squire 1937, Taylor 1936, Williams 1924). In addition to nutrients, agar, carboxymethylcellulose and/or sodium alginate were added to stabilize and solidify the media, making them firm and homogeneous without free water. The carrot medium used in fruit fly rearing (Finney 1956) was also tried.

To prepare the media, the agar, dissolved in the required amount of hot distilled water, was added to the weighed dry constituents, with the exception of carboxymethylcellulose, and blended until homogeneous. Carboxymethylcellulose was added last since even such low concentrations as 0.5% to 1% caused the media to become very viscose, and hence difficult to blend.

Aseptic techniques were used throughout the experiments conducted to develop dietary media. Either 2 to 3 cc. of media were placed in 15×150 mm. culture test tubes, or 15 to 25 cc. in 125- or 250-ml. Erlenmeyer flasks, and the vessels plugged with nonabsorbent cotton. The media were then autoclaved at 20 pounds pressure for 15 minutes and held for 2 or 3 days at 28° C. to allow any free water to dissipate.

Sterile media were inoculated by introducing 4- to 5-day-old eggs or 1-day-old first-instar larvae from the sterile egg storage vials by means of sterile camel's-hair brushes at the rate of 1 to 5 eggs or larvae per culture tube, or 10 to 30 per Erlenmeyer flask.

Antimycotic Screening.—High fungous contamination presented a difficult problem for it was not uncommon to have five or more species of mold per culture tube, mostly in the genera *Aspergillus* and *Rhizopus*. Two possible solutions to this problem were the use of aseptic techniques or chemical control, the former being time-consuming and not practical in a large-scale rearing technique. Therefore, a series of experiments were conducted using antimycotic agents alone, or in combination with certain portions of the aseptic technique, to suppress or destroy fungi and yet allow normal development of the larvae. The procedures described above to develop artificial media were followed, with the exception of such aseptic techniques as autoclaving the medium and sterilizing the eggs. Such precautions as sterilizing the glassware and other equipment when possible, maintaining a clean, dust-free working area, and covering the culture tubes and flasks were taken to reduce contamination. Twenty-two compounds were screened at levels from 0.01% to 2% by blending them with the other constituents in the cottonseed meal diet developed in earlier experiments. Alcoholic stock solutions of chemicals with low aqueous solubility were prepared (10% to 15% in 95% ethanol w/v) and the calculated volume, to give the required concentration, was added to the medium. These agents were evaluated for effectiveness on the basis of whether they inhibited fungous growth for 10 days, after which time the larvae

were capable of competing with fungi without appreciably affecting larval growth and development. A minimum of 10 replicates of at least 8 flasks or 50 culture tubes per replicate were used at each concentration. No attempt was made to inoculate the media with fungous spores; any fungous development came from normal contamination.

Preliminary Mass-Rearing Experiments.—To determine the feasibility of mass rearing the larvae, 10 to 50 eggs or larvae were placed on the cottonseed-meal medium containing antimycotic compounds in petri dishes or 125- or 250-ml. Erlenmeyer flasks. The rearing of many larvae per vessel introduced the problem of cannibalism (Taylor 1936); two possible solutions were offered by (1) providing a large area of media per larva, and (2) segregating the larvae within the flask on the premise that once the larvae were settled cannibalism would be greatly reduced. Segregation as a solution is demonstrated in nature since as many as 18 to 20 larvae may be found in one boll with only the septa, seed coat, and cotton to separate them.

To provide a large area per larva, hot medium was poured into the vessels to yield a flat mass about one-fourth inch deep. Attempts to segregate the larvae within the vessels were carried out as follows: By using 20 to 40 ml. of medium in the form of 1-ml. cubes or balls, or 2- to 3-cm. lengths of soda straws filled with medium; by creating physical barriers to separate the medium into small units by pouring it into sterile metal bee-comb foundations, nonabsorbent cotton, pieces of paper, folded cardboard, or plastic ice trays (½-inch cube type); or, by placing a layer of nonabsorbent cotton on the surface of a flat mass of medium.

To curtail wandering of first-instar larvae, all deep containers and some shallow containers were provided with places for these insects to conceal themselves. Absorbent cotton and sterile filter paper were placed in the containers to absorb the condensate.

No aseptic techniques were used in these experiments, but the precautions taken in screening antimycotic compounds were followed. Both sterilized and nonsterilized eggs were used. Of the nonsterilized group, either free-washed eggs or the eggs on the oviposition site were introduced on the medium.

All procedures involving preparation of media were conducted in a clean laboratory at 27° C. and 60% relative humidity. Rearing was carried out in a temperature-controlled insectary at 28°, 65% to 75% relative humidity, and with 25 foot-candles of illumination for 10 hours per day.

RESULTS AND DISCUSSION.—More than 90% of the nonsterilized eggs hatched. The use of Stride's technique for egg sterilization resulted in an egg mortality which increased with the pretreatment age of the egg—that is, about a 20% mortality of 1-day-old eggs and a 40% mortality of 4-day-old eggs. Under laboratory conditions, the hatch of nonsterilized eggs was better than 70% in a relative humidity of 10% to 100%, and better than 90% in a relative humidity of 70% to 90%.

Plant Materials.—All of the natural plant materials except intact okra pods were unsatisfactory as diets. Sprouted cottonseed had an insignificant infestation (0.1%), and the other materials had none. Fungous contamination was great. From a total of 9,117 eggs and 362 okra pods (approximately 50 replicates), 315 pink bollworms were recovered; thus, 3.5% of the eggs resulted in an average infestation of 0.9 larva per pod. In the best experiment, 653 eggs placed on 43 pods yielded 64 pink bollworms (9.7% of the eggs) and an average of 1.6 larvae per pod. There was little fungous contamination. Of the okra pods of different ages, those that were too old and fibrous for human consumption yielded the best results, since they possessed seeds which were mature but not hard and pods that did not have an excessive amount of sap. The exudate from entry holes on younger pods trapped many newly hatched larvae.

Two groups of experiments were conducted using the sprouted-cotton-seed technique developed by Vanderzant *et al.* (1956). In the first group, no larvae were recovered from 40 tests involving a total of approximately 6,000 eggs and 15,000 sprouted cottonseeds. In the second group involving 12 tests, 71 pink bollworms were recovered from a total of 5,215 eggs and 4,096 sprouted cottonseeds, which amounted to 1.4% of the eggs and averaged 1.7 larvae per 100 cottonseeds. In the best experiment of this group which involved 500 eggs and 650 seeds, only 19 pink bollworms were recovered (3.8% of the eggs). Fungi covered the cottonseed in spite of the fungistatic treatment. Pink bollworm-infested seed were dipped into the antimycotic solution without causing undue mortality among the larvae. The fungicidal solution of copper sulfate was used in only one experiment, but it compared favorably with the treatment using *n*-butyl-*p*-hydroxybenzoate. No cannibalism was observed, but the newly hatched larvae began to die after a few hours if they had not entered the seed mass. The placement of sprouted seed over hatching eggs did not noticeably increase the infestation. Some bacterial and/or fungous breakdown of the seeds occurred, ultimately resulting in a rotting mass, but some healthy insects were recovered from the fungous-decomposed seeds.

Okra pods have an advantage over cottonseeds in that fungous contamination is low and larvae can be extracted from them easily; however, the major disadvantage of okra is that cheap pods are not available in large quantities throughout the year.

Commercial Plant Products.—Soybean flour, ground carrots, and sesame seed, in the combinations used in these experiments, proved unfavorable. It was found that the carrot diet developed by Finney (1956) for tephritid fruit fly rearing would support the growth of the pink bollworm, but the larval developmental time was approximately twice the average period and the recovery was low (10% of the introduced eggs). Mature larvae fed on this diet were pale with little pink color and weighed about 25% more than larvae raised on cotton. The adults were lighter colored and also about 25% larger.

Twenty mixtures using cottonseed meal or similar seed products as bases and two using peanut flour as a base, in combination with other nutrients, were tried. Of these experimental media, the two most successful are shown in table 1. Brewers' yeast must be added to these two diets even though the bases, particularly cottonseed meal, are very high in nutritive value. No mature larvae were recovered from diets without yeast. The water content was

Table 1.—Diets for rearing pink bollworms using peanut flour and cottonseed meal.

INGREDIENT	PEANUT FLOUR DIET (GM.)	COTTONSEED MEAL DIET (GM.)
Flour or meal	14	10
Agar	2	2
Albumin	—	1
Brewers' yeast	2	2
Carboxymethylcellulose[a]	1	2
Cholesterol	—	0.2
Cottonseed oil	4	—
Dextrose	2	2
Distilled water	75[b]	57[b]

[a] Or sodium alginate for cottonseed meal.
[b] Milliliters.

very important for optimal growth; if it dropped much below 70%, the larvae had a tendency to slow their feeding activities; if it dropped below the 40% level, the larvae ceased feeding. When these larvae were transferred to fresh medium they resumed feeding and completed their life cycle. This phenomenon was observed in larvae found in field-collected, dry cottonseeds; for when seeds were moistened, the larvae resumed feeding. Also, if they were removed from the dry seeds to artificial medium, they would resume feeding and complete their life cycle. Uncovered cottonseed-meal medium lost about 25% of water in 14 days under laboratory conditions.

The developmental rates, larval, pupal, and imaginal sizes, and fertility of the pink bollworm reared in these media were within the range of variations of the wild population. The larvae reared on peanut-meal medium were, in general, very pale and some even had a greasy, grey appearance.

The survival rate of the larvae fed on these two diets depended on the container, the amount of free water (condensation), and the number of eggs or larvae placed in each container. Larvae raised at the rate of one per culture tube had a low mortality (10% to 15%); for the most part death occurred when first-instar larvae drowned in droplets of water or starved owing to their straying from the media. When more than one individual was placed in a tube, cannibalism caused the greatest loss. Seldom did more than one larva survive; the survivor probably received a developmental boost from feeding on its fellow larvae. The mortality rate of flask-reared larvae was higher per flask as the number of introduced eggs or larvae was greater.

When fungous contamination was present, the mortality rate rose in proportion to the extent of contamination. Usually, if larvae were 8 to 12 days old before extensive contamination appeared, they were well enough developed to complete their life cycle, which indicated that survival at first was a matter of competition with the fungi. However, one species of *Aspergillus* was noxious since all larvae in the container were killed when it was present in appreciable amounts. Whether death was brought about by the abundance of fluid produced by the mold or by the mold itself was not determined. When extensive fungous contamination was present, older larvae ingested spores and mycelia with no ill effects. This was quite evident when the spores were black since the larval intestinal tracts were visibly darkened, at times to the point where the entire larva seemed black.

Although bacterial contamination was present at times, it was not a problem. An occasional, sour-smelling bacterial contaminant liquified the media thereby destroying the culture.

Antimycotic Screening.—The fungicides and fungistatic agents that gave no practical control, either because they were not effective against fungi or were effective only at concentrations that were toxic to the pink bollworm, were Anticarie (hexachlorobenzene), Dyrene (2,4-dichloro-6-(*o*-chloroanilino)-*s*-triazine), a captan analog (methylendomethylene captan), chloranil, copper chloride, copper sulfate, dichlone, ferbam, pentachloronitrobenzene, sodium dimethydithiocarbamate, streptomycin, terramycin, thiram, ziram, and zineb.

The effective antimycotic chemicals were Butoben (*n*-butyl-*p*-hydroxybenzoate), captan, formaldehyde, methylparaben (methyl-*p*-hydroxybenzoate), potassium sorbate, sodium benzoate, sodium propionate, and sorbic acid. In concentrations of 0.8% to 2% most of these compounds inhibited at least 80% of fungous contamination but retarded larval growth, increased developmental time, and caused larval mortality. These adverse effects increased when concentrations of compounds were increased. Formaldehyde at 0.10% inhibited fungous growth, but this provided too small a safety margin between the nontoxic and toxic fungistatic concentration (0.17%). Butoben and methylparaben in concentrations of 1.6% to 2% brought about as much as a 50% larval mortality, increased the rate of development by five times, and affected larval growth so that pupae and adults were about one-third the normal size. Below 0.2% these compounds had no effective fungous control.

Of these compounds, sorbic acid, which has been used approximately 3 years at this laboratory, and potassium sorbate showed promise when used singly at concentrations of at least 0.8%. Potassium sorbate at 0.8% or better controlled mold in 85% of the culture tubes for 15 to more than 90 days with a larval recovery of 72%. However, the larval period was increased approximately 50%. The aqueous solubility of potassium sorbate gave it an advantage over sorbic acid (soluble in alcohol).

Approximately 130 combinations of these compounds were tried; the most effective contained Butoben or methylparaben, sodium benzoate or sodium propionate, and sorbic acid or potassium sorbate at concentrations of 0.2% to 0.4% each. The combination of antimycotic agents adopted was Butoben (0.2%), sodium benzoate (0.2%), and sorbic acid (0.3%). This combination inhibited fungous growth in at least 80% of the cultures without detectable adverse effect on the larvae for 8 days. The larval recovery was better than 90% and the larval period was increased 18%.

Preliminary Mass-Rearing Experiments.—The use of the cottonseed medium, shown in table 1, with the addition of the three fungistatic compounds, Butoben (0.2%), sodium benzoate (0.2%), and sorbic acid (0.3%), was adequate for mass rearing. It was found that nonsterilized eggs could be used although the chances of introducing fungous contamination were much greater than when

sterilized eggs were used. When the oviposition site with the eggs was placed on the medium, the initial contamination was greatly increased, with the result that when the fungistatic agents began losing their effectiveness, mold took over rapidly, reducing the fungi-free period by about 20%. This source of contamination was alleviated somewhat by lightly wrapping the oviposition site and its eggs in sterile absorbent cotton.

The results of experiments conducted to thwart the cannibalistic tendencies of the pink bollworm showed that both the space and segregation approaches could be used. Eight to ten pink bollworms were reared from eggs to adults in an Erlenmeyer flask if approximately 1 square inch or 0.5 cubic inch of medium was allowed per worm. Ten to twenty larvae were reared in containers in which the larvae were segregated by means of small units of medium or physical barriers. Cut straws, fragments of bee-starter frames, and similar objects forming physical barriers were useful but not practical because they were difficult to fill with medium and later wash. Cubes or balls of medium entailed labor which might prove uneconomical in raising large numbers of insects. Another disadvantage of this technique of using small units was that large surfaces were created which allowed more rapid drying and decomposition of antimycotic agents and a greater possibility of fungous contamination.

Plastic ice trays were filled about half full of medium; eggs were evenly distributed over the medium, and the trays were wrapped with household aluminum foil or parafilm to keep the larvae close to the medium. There was a yield of 10 to 20 larvae per tray. The objections to these trays were that their large area was a source for much fungous contamination and that filling and washing them were time-consuming. By pouring medium into folded cardboard, pieces of paper, or absorbent cotton which were sterile, physical barriers were created similar to those found in a boll, larval movement was deterred, and each 125-ml. flask yielded 10 to 20 larvae.

The loss due to wandering of the first-instar larvae was as much as 100%; therefore, it was important to keep them in close proximity to the medium. Providing places of concealment to deter larval movement was not too satisfactory. The preceding experiments using barriers were observed for a decrease in larval movement, but no appreciable differences were noted, nor did placing absorbent cotton on the surface of the medium make a noticeable difference. However, the cotton did reduce the condensation which was a major cause for the loss of many wandering larvae, and filter paper also aided in this respect. The objection to cotton or filter paper was again the increased contamination that resulted from the use of these materials. Confinement curtailed wandering and reduced the loss of larvae at least 80%. Opaque covers such as foil were objectionable because the larvae could not be observed.

Petri dishes, because of their size and shape, had several favorable attributes. They could be stacked easily, thereby making maximum use of storage space; they were shallow enough so that the first-instar larvae were close to the medium; and they were easily handled. The halves of the average petri dish did not fit tightly enough to prevent the first two instars of the larvae from escaping, but several successful methods of sealing these halves were found. The lip of the lower half of the petri dish could be sealed against the upper half by means of a gasket of cotton, filter paper, or blotting paper. Another method tried was that of pouring the medium into the top half and pushing the halves together, thus sealing them with a layer of medium. Also, the prepared petri dishes could be assembled and absorbent cotton packed closely between the edges to prevent the larvae from escaping.

The most satisfactory method found for preparing petri dishes consisted of filling about three-quarters of the space within the top half of each with medium, inoculating the medium with eggs, filling the remaining space with absorbent cotton to help absorb moisture and to act as a barrier, and sealing the two halves with the layer of medium, as described above.

It was found that the boll weevil (*Anthonomus grandis* Boh.) and a humpbacked fly, *Megaselia* sp. (Phoridae), could be raised on the cottonseed-meal medium. Growth and development of the boll weevils were slow and sporadic. Phorid flies, with a life cycle similar to that of Drosophila, were reared simply and rapidly, and several generations could be raised on the same lot of medium. Fungous contamination was no problem owing to the fungistatic agents. The larval phorid flies appeared to create conditions that also inhibited fungous growth.

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The Metabolism of C^{14}-Labeled DDT in the Larvae, Pupae, and Adults of *Drosophila melanogaster*[1]

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ABSTRACT

A metabolic product of C^{14}-DDT in *Drosophila melanogaster* Meigen was found to be primarily 2,2,2-trichloro-1,1-bis-(*p*-chlorophenyl) ethanol, (Kelthane®), confirming the results of Tsukamoto. Kelthane was found in larvae, pupae and adults exposed to larval medium containing C^{14}-DDT and also in adults exposed by topical application to C^{14}-DDT. Of the two strains of *Drosophila* examined by adult topical exposure, one (Oregon Rc) showed the active formation of Kelthane, while the other (Oregon R) did not. A new, unknown metabolite of the same chromatographic mobility as *p,p'*-dichlorobenzophenone was found in the larval exposure as well as a large amount of a very polar metabolite(s). Another new metabolite of the same chromatographic mobility as 2,2-dichloro-1,1-bis-(*p*-chlorophenyl) ethanol was found in the internal tissue extract upon adult topical exposure.

The metabolism of DDT has been the subject of research in this laboratory for a number of years. We have been particularly interested in the comparative biochemistry of DDT metabolism in different species of DDT-tolerant and nontolerant insects. From this view, it is particularly interesting to note that Tsukamoto (1959) has reported the occurrence of Kelthane® (2,2,2-trichloro-1,1-bis-(*p*-chlorophenyl) ethanol) as the primary metabolite of DDT in *Drosophila melanogaster* Meig. Tsukamoto has demonstrated Kelthane in the larval medium, pupae, and emerged adults of cultures treated with DDT in the larval medium. We wish to confirm the work of Tsukamoto and to report some additional observations which we have made.

METHODS AND MATERIALS. I. *Larval Exposure.*—Ten parts per million of C^{14}-DDT were incorporated in the larval medium of pumpkin. Forty adult male and female *Drosophila* of the Oregon Rc strain described below were introduced into each of six 37-mm.×120-mm. test tubes containing approximately 1 gram of the DDT-containing medium. The parental generation had been raised on a pumpkin medium containing 10 p.p.m. of nonradioactive DDT.

Extraction Procedure for Larval Exposure.—After the emergence of the first filial generation, the extraction procedure was started as follows:

A. Internal Extract of Live Adults.—Approximately 100 live adult flies were collected from the cultures. The flies were anesthetized with carbon dioxide, rinsed with 10 ml. acetone for approximately 1 minute, and the rinses reserved and combined with extract of larval medium below. The flies were then homogenized three times in 5-ml. volumes of acetone containing a pinch of anhydrous sodium sulfate, and centrifuged; the supernatants were collected as the internal extract. After concentration to a small volume under a dry nitrogen stream, the extract was purified by the procedure of Menn *et al.* (1960) using 100% acetonitrile by descending elution from a 1½-inch-wide strip of Whatman No. 2 filter paper. The purified extract was evaporated just to dryness and taken up in a small volume of acetone. The acetone solution was spotted on a strip of Whatman No. 2 filter paper 1 inch wide and approximately 20 cm. long. Chromatography of the extract was performed on this filter paper using the procedure of Gordon (unpublished) in which a stationary phase of β-methoxypropionitrile (10% v/v in acetone) was coated on the paper and the chromatogram was developed by descending elution with the di-*iso*propyl ether saturated with the stationary phase, β-methoxypropionitrile (referred to hereafter as I/M system). The results are illustrated in figure 1.

B. Extract of Larvae and Pupae.—The remaining larvae and pupae were removed from the culture medium. About 120 larvae and 20 pupae were found, rinsed with acetone and subjected to the same tissue acetone extraction as above for adult tissue extraction. The larval and pupal extract was chromatographed in the I/M system as above. The results are not illustrated, but were very similar to figure 1.

C. Extract of Larval Medium.—The remaining material in the culture tubes, after collection of the live adults, larvae, and pupae, was combined, extracted three times by homogenization with 25-ml. volumes of chloroform containing a large excess of anhydrous sodium sulfate, and centrifuged; the supernatants were combined as the medium extract. No attempt was made to segregate the

[1] Contribution from the Laboratory of Insect Physiology and Toxicology, Department of Entomology and Parasitology, University of California, Berkeley.

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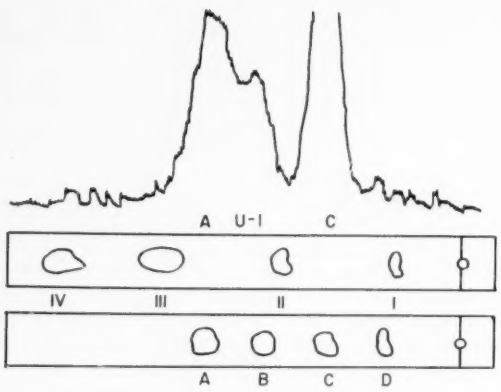

FIG. 1.—Internal extract of live adults from the larval exposure method, rechromatographed (3 times) in I/M system. I, II, III and IV are reference dyes. A: DDT; B: *p,p'*-dichlorobenzophenone; C: Kelthane; D: 2,2-dichloro-1,1-bis-(*p*-chlorophenyl) ethanol (FW-152); U-I: Unknown I.

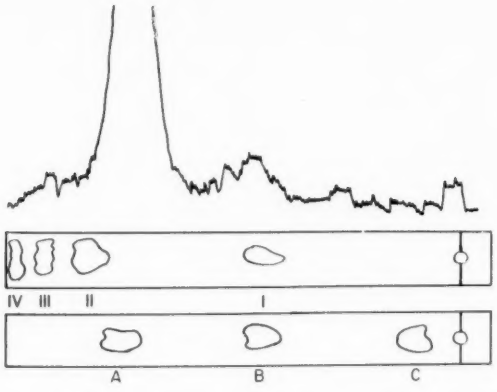

FIG. 2.—Kelthane-like group from the extract of the larval medium of the larval exposure method rechromatographed in the C/M system. I, II, III and IV are reference dyes. A: Kelthane; B: 2,2-dichloro-1,1-bis-(*p*-chlorophenyl)ethanol (FW-152); C: DDA.

dead adults of the parental generation from the medium; hence, this extract represents the metabolism of the adult parental generation, the excreted products of the larvae and the first filial generation pupae, and the adults which died prior to collection. Purification was performed by evaporating the initial chloroform extract just to dryness, taking up in 25 ml. of low boiling petroleum ether, and successively extracting the petroleum ether fraction three times with an equal volume of 100% acetonitrile. The acetonitrile extract was spotted on a filter paper strip and purified by the Menn acetonitrile method with 100% acetonitrile. The purified extractives were separated by chromatography in the I/M system described above. Figure 2 illustrates the results obtained after separation by additional chromatography (see extraction procedure for adult exposure for detailed discussion).

II. *Adult Topical Exposure.*—Two strains of *Drosophila* were examined by topical application. Oregon R flies were obtained from the California Packing Corporation. This strain had previously been reared in our laboratory from a genetically pure stock obtained from the Department of Zoology and had since been maintained by the California Packing Corporation. Another strain known as Oregon Rc was obtained from the Department of Genetics where it had been maintained continuously from a variant of the original Oregon R stock. All of the strains used in these experiments were maintained in our laboratory on a medium of sugar-water mixed with canned pumpkin. Adults were collected 4 days after emergence and anesthetized with nitrous oxide. One thousand adult male and female flies collected from the Oregon Rc colony (Rc flies)were treated topically with an acetone solution containing 0.04 μg. of C^{14}-DDT/$\frac{1}{2}$ μl.; 400 adults collected from the Oregon R colony (R flies) were treated topically with an acetone solution containing 0.03 μg. of C^{14}-DDT/$\frac{1}{3}$ μl. The mortalities from this dosage were: Oregon Rc flies, 96% mortality in 16 hours; Oregon R flies, 90% mortality in 14 hours.

Extraction Procedure for Adult Exposure.—Live flies were segregated from the dead or moribund flies. Both groups were rinsed with 10 ml. of acetone for approximately 1 minute, and the rinses reserved. The individual groups were then homogenized three times with 5-ml. volumes of acetone containing a pinch of anhydrous sodium sulfate, and centrifuged; the supernatants were collected. Extracts from live flies are denoted as internal extracts of live flies; extracts from the dead and moribund flies are denoted as internal extracts of dead flies. The holding vessels were scrubbed with acetone, and this acetone was combined with the external acetone rinses to give the combined external-jar extract. All of the extracts were purified by the Menn acetonitrile method and were concentrated under a dry nitrogen stream for chromatography. In general, the di-*iso*propyl ether/β-methoxypropionitrile system (I/M) described above was used; however, where noted in the figures, other systems were used for better resolution of the components. These chromatographic systems were prepared according to the procedure of Gordon (unpublished) and as described for the di-*iso*propyl ether/β-methoxypropionitrile system as above. The alternate systems are as follows:

a. Cyclopentane/β-methoxypropionitrile (C/M), where the cyclopentane, saturated with β-methoxypropionitrile, was substituted for the di-*iso*propyl ether phase, the β-methoxypropionitrile phase being prepared as above;

b. Di-*iso*propyl ether/glutaronitrile (I/G), where the di-*iso*propyl ether phase was saturated with glutaronitrile and where a 13% (v/v) acetone solution of glutaronitrile was substituted for the β-methoxypropionitrile phase as the coating for the filter paper.

III. *Methods of Detection and Materials.*—The distribution of radioactivity along the chromatogram was determined by scanning the chromatogram with a windowless end flow Geiger-Müller tube and a recording ratemeter as described by Hoskins & Witt (1958). The distribution of chlorine-containing materials was determined by the procedure of Mitchell (1956). Mitchell reagent was sprayed on the developed chromatogram after scanning

for radioactivity and after blueprinting. The blueprinting procedure used was that of Gordon (1958) and represents the distribution of the ultra-violet-absorbing materials on the chromatogram.

The radioactive DDT used in this study was prepared in this laboratory and was shown to have only one component chromatographically in all of the systems used. Kelthane was supplied to us by the Rohm & Haas Company, Philadelphia, Pennsylvania, and was shown to have only one component in all of the chromatographic systems used.

RESULTS AND DISCUSSION.—*Larval Medium Exposure.*—As was noted above, the results of Tsukamoto (1959) from similar larval exposure experiments were confirmed. The internal extract of live adults illustrated in figure 1 clearly shows radioactive materials of the same mobilities as DDT and Kelthane. An unknown material is also indicated near the mobility of DBP (*p,p'*-dichlorobenzophenone), which was not reported by Tsukamoto. The extract of larvae and pupae also showed the occurrence of DDT and Kelthane, but not that of the unknown metabolite I of the internal extract of live adults.

In the course of the analysis of the larval medium extract, it was convenient, because of the high amount of radioactivity present, to isolate first the DDT-like group (that material at the same mobility of DDT or higher) and the Kelthane-like group (that material having a mobility below that of DDT) from an initial I/M chromatogram, which is not shown. The initial chromatogram, divided as above, was eluted, with acetone, the eluants concentrated under a dry nitrogen stream and spotted on two second chromatograms, which were developed by the I/M system and the C/M system, respectively, as described above. The Kelthane-like group is shown in figure 2, which indicates Kelthane and a material of the same mobility as 2,2-dichloro-1,1-bis-(*p*-chlorophenyl) ethanol (FW-152). This procedure is very effective in purifying the extracts and improving the resolution of the components. It is not felt that this procedure is likely to introduce any artifacts.

Adult Topical Exposure.—Two distinct types of metabolism were observed in the adults of the two strains examined. The Oregon Rc strain produced considerable quantities of Kelthane in the internal extract of dead flies, as shown by figure 3a. DDT, Kelthane, and a FW-152-like material were observed as well as a polar metabolite of unknown character. Of the metabolites separated, only Kelthane has been identified. The radioactive area, in which Kelthane should occur in figure 3a, was cut out of the rest of the chromatogram and was eluted with acetone. After concentration, the metabolite was mixed with 10 μg. of nonradioactive Kelthane (needed to give a positive Mitchell test) and was spotted on paper and developed in the C/M system. The distribution of radioactivity along the chromatogram is illustrated in figure 4a, the distribution of ultra-violet-absorbing materials in figure 4b, and the distribution of the chlorine-containing materials in figure 4c. In all of the tests employed, the radioactive material coincided with the added nonradioactive Kelthane. While this evidence is not complete for the exclusive identification of the metabolite as being Kelthane, we feel that it is more than adequate in the light of Tsukamoto's exhaustive chemical characterization.

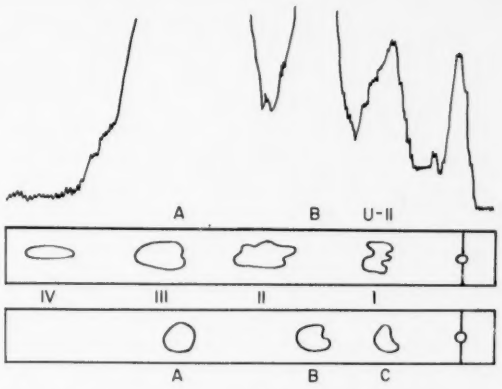

FIG. 3a.—Internal extract of 950 dead Rc flies after 17 hours of exposure in the I/M system.

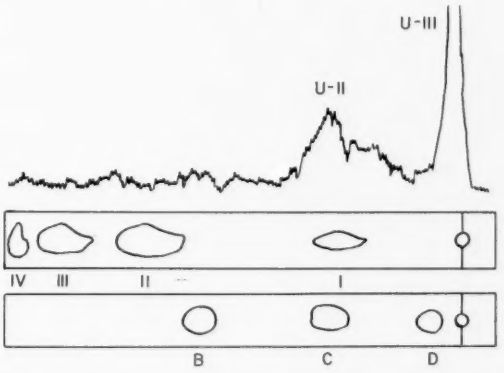

FIG. 3b.—Unknown II from 3a. chromatographed in C/M system. I, II, III and IV are reference dyes. A: DDT; B: Kelthane; C: 2,2-dichloro-1,1-bis-(*p*-chlorophenyl)ethanol (FW-152); D: DDA; U-II: Unknown II; U-III: Unknown III. (Symbols refer to both figures.)

Figure 3b illustrates that a second group of metabolites besides Kelthane is formed in the adult. Two materials are clearly seen: an FW-152-like material (Unknown II) and a polar material(s) (Unknown III).

Similar results, not shown, were found in the internal extracts of the live flies. However, the amount of activity was too small for adequate testing.

While Kelthane is a definite metabolite of DDT in Oregon Rc adults, it did not appear in the metabolism of DDT in adults of the Oregon R strain. The radioactive components of the extracts of the topically treated Oregon R adults were chromatographed in the I/M system. No Kelthane was detected. While the Oregon Rc strain was derived from the Oregon R strain a number of years ago, the two strains differ in their tolerance to DDT (Oregon Rc topical LD-50 = 0.06 μg./fly; Oregon R LD-50 = 0.03 μg./fly).

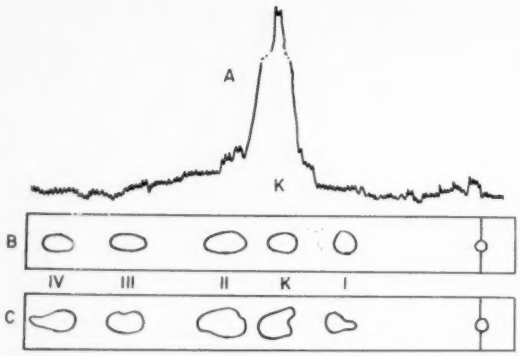

FIG. 4.—Identification of Kelthane from figure 3a. (Ten μg. nonradioactive Kelthane added before chromatography in C/M system.) A. Radioactivity; B. Ultra-violet adsorbant materials; C. Chlorine containing materials by Mitchell test; I, II, III and IV are reference dyes; K: Kelthane.

Conclusion.—From the larval exposure experiments, it may be concluded that the larvae and pupae produce Kelthane as the primary metabolite in their body tissues. Adults developing from larvae exposed to DDT also contained Kelthane as the major metabolite. From these experiments it cannot, however, be definitely concluded that the adult organism is or is not capable of Kelthane formation. The Kelthane found in the adults could be a carryover from the Kelthane formed in the larval stage or absorbed from the Kelthane found in the medium. Kelthane probably is not the final metabolite of DDT in the larvae, since a more polar material(s) has been detected. A material of the same chromatographic mobility as FW-152 has definitely been detected as a minor metabolic product. A large quantity of very polar material has also been detected. The exact identity of these products is still unknown.

The adult exposure experiments indicate that the ability to form Kelthane from DDT persists in the adult stage of some strains of *Drosophila*. The presence of Kelthane found in the adult organisms developed from DDT treated larvae is most probably explained as the result of the active metabolism of DDT to Kelthane in the adult stage. However, storage from the larval stage or absorption from the medium are still not excluded as possible sources of Kelthane.

While the ability to form Kelthane from DDT appears in the adult stage of some strains of *Drosophila*, the extent of this ability varies from strain to strain and appears to be absent in the Oregon R strain. The higher rate of formation of Kelthane by the Oregon Re strain is in line with its higher DDT tolerance.

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Laboratory and Field Studies on Orchard-Mite Resistance to Kelthane[1]

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ABSTRACT

In September of 1958, a few apple growers in Washington reported the failure of Kelthane® (1,1-bis(*p*-chlorophenyl) 2,2,2-trichloroethanol) in controlling the McDaniel mite, *Tetranychus mcdanieli* McGregor. During 1959, this problem occurred in many other apple orchards in north central Washington.

When difficulties were first reported, field studies indicated that timing, application, and the amount of active material used were not responsible for the failures, but rather that the mites had developed a resistance. Several other chlorinated hydrocarbon acaricides also failed to control the mites. Laboratory studies confirmed the presence of Kelthane-resistant strains of the McDaniel mite at several locations. One strain from an orchard where control had been especially difficult showed a 200-fold increase in the LD-50 over a susceptible strain.

Laboratory studies with strains of the two-spotted spider mite, *Tetranychus telarius* (L.), from different areas showed that they were about equally susceptible to Kelthane.

After several years of research and extensive orchard testing, Kelthane (1,1-bis(*p*-chlorophenyl) 2,2,2-trichloroethanol) received a tolerance and was available for general orchard use for mite control during the 1958 season. The material gave excellent control of several species of phytophagous mites, and was widely accepted by fruit growers in Washington. Late in the summer of 1958 a few reports of difficulty in controlling the McDaniel mite, *Tetranychus mcdanieli* McGregor,[4] with Kelthane were received. It was then felt that most of this difficulty was caused by poor application or by applying

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[4] Mites identified by Edward W. Baker.

Table 1.—The effect of early season sprays applied by handguns on the buildup of McDaniel mites on apple trees. Orondo, 1959.

Material[a]	Rate per 100 Gallons	Average Number of Mites per Leaf on Each Sampling Date: Prespray May 29	June 10	June 18	June 29	July 13
Kelthane, Flowable (50% technical)	0.75 pt.	0	1.7	2.2	11.2	24.5
Tedion 25%, WP + KT7N	0.5 lb. 4 oz.	.8	1.3	1.7	3.2	2.1
Tedion 25%, WP + Oil, Volck Supreme	1 lb. 0.5 gal.	.5	.2	.5	.2	1.2
Dimite, 2 lbs. per gal. EC	1.5 pts.	2.2	.5	.9	3	7.7
Genite 50%, WP	1 lb.	.3	.2	1.2	2.8	23
Check (unsprayed)		.2	1.2	5	4.1	19

[a] WP, wettable powder; EC, emulsion concentrate.

the material when heavy populations were present. However, preliminary tests were begun in September, 1958, in one orchard at Orondo, Washington, and in the laboratory to determine the cause of the difficulty. Some evidence of a possible resistance was observed in these tests.

As the 1959 season progressed, additional reports of difficulty in controlling the McDaniel mite with Kelthane were received. These came from a large area, including the Wenatchee River Valley, Wenatchee, Orondo, Entiat, and the Methow Valley apple-growing districts. Some growers reported that two or even three applications during the 1959 season had failed to control these mites. The control failures seemed to be restricted to the McDaniel mite in apple orchards even though this species occurs on other deciduous fruit crops (Nielsen 1958).

FIELD STUDIES.—*Materials, Methods and Results.*—Tests were conducted to evaluate substitute materials for Kelthane as well as to determine the reasons for the lack of control. Several other chlorinated hydrocarbon acaricides and organophosphorus compounds were tested. However, only the chlorinated hydrocarbons are included here for comparison. Two orchards of mixed Winesap and Delicious apple trees were selected for these studies, one located at Orondo, Washington, and the other at Entiat, Washington. In all tests, the treatments were evaluated by mite counts made from leaf samples taken at random from the lower portion of the trees. The samples were taken to the laboratory and the leaves brushed with a mite brushing machine (Henderson & McBurnie, 1943). The live mites were then counted under a dissecting microscope.

The first four tests were conducted in the orchard at Orondo. The grower reported that he first applied Kelthane during July of 1958 and obtained successful control. The second application, applied during August of 1958, failed to control the mites. The third application was the preliminary test conducted by the authors in September, 1958. It also failed to control the mites.

Test No. 1—Orondo.—Single tree plots replicated four times in a randomized block design were established. The trees were sprayed on June 2, 1959, with conventional high pressure equipment and orchard guns at 600 p.s.i. using 20 gallons of spray per tree. Twenty-five leaves per replicate were sampled on each date (table 1). The sprays were applied when the populations on the trees were low. However, even under these conditions Kelthane and Genite[6] (2,4-dichlorophenyl benzenesulfonate) were not effective, and mite populations built up as rapidly on trees sprayed with these materials as they did on the unsprayed check trees (table 1). Dimite[6] (4,4′-dichloro-*alpha*-methylbenzhydrol) suppressed the mite population to some extent. Tedion (2,4,4′,5-tetrachlorodiphenyl sulfone) plus Volck Supreme oil and Tedion plus KT7N,[5] a surfactant, provided good control.

Test No. 2—Orondo.—One-half acre plots replicated twice (Kelthane plus KT7N had only one replicate) were set up and sprayed on June 4, 1959, with air carrier equipment at a semiconcentrate dosage (2×) using 500 gallons per acre. Fifty leaves were picked at random from each replicate on each sampling date (table 2). Again, the spraying was done prior to the buildup of large numbers of mites (table 2). All materials provided some suppression of the mite populations over those found on the unsprayed check trees. Plots treated with Kelthane and Kelthane plus the surfactant KT7N, required re-treatment in mid-July.

The plot treated with Kelthane plus Z-1,[5] a surfactant, had a low population at the final count. Although formal counts were not made in this plot after July 13, a rapid increase in the mite population owing to egg hatch made re-treatment necessary on July 20. The one application of Tedion gave seasonal mite control even though the surrounding trees had heavy mite populations and showed mite injury before mid-summer.

Test No. 3.—Orondo.—Since the materials in Test No. 2 were sprayed at a semiconcentrate dosage, an additional test was carried out to determine if a more

[5] The principal functioning agents of KT7N and Z-1 are sodium sulfonates and combined fatty acids.

Table 2.—The effect of early season sprays applied at a semiconcentrated dosage by air carrier equipment on the buildup of McDaniel mite populations on apples. Orondo, 1959.

Material	Rate per 100 Gallons	Average Number of Mites per Leaf on Each Sampling Date: Prespray May 29	June 10	June 18	June 29	July 13	Aug. 20
Tedion 25%, WP	2 lb.	0.2	0.1	0	0.1	0.6	0.4
Kelthane 18.5%, WP	3 lb.	0	.4	.9	3.4	13.3	
Kelthane 18.5%, WP +Z-1	3 lb. 4 oz.	.8	.1	0	1.1	1.4	
Kelthane 18.5%, WP +KT7N	3 lb. 4 oz.	0	0	.4	.5	7.0	
Check (unsprayed)		3.9	.6	1.3	7.6	32.5	

Table 3.—The control of the McDaniel mite on apples by summer applications of chlorinated hydrocarbon acaricides. Orondo, 1959.

Material	Rate per 100 Gallons	Average Number of Mites per Leaf on Each Sampling Date: July 21	July 27	August 3	August 10
Dimite, 2 lbs. per gal. EC	1.5 pt.	67	56	4	24
Kelthane, 40W (40% technical)	1.5 lb.	10	14	11	—
Chlorobenzilate 25%, WP+X77[a]	1.5 lb. 1.75 oz.	28	14	19	—
Check (unsprayed)	—	14	25	96	—

[a] The principal functioning agents of X77 are alkylarylpolyoxyethylene glycols, free fatty acids and isopropanol.

Table 5.—Control of the McDaniel mite on apples with summer applications of acaricides. Entiat, 1959.

Material	Pounds per 100 Gallons	Average Number of Mites per Leaf on Each Sampling Date: Prespray July 2	July 13	July 20	July 27
Kelthane, 40W (40% technical)	1	13	17.7	34	
Kelthane, 40W	2	19	10	34	29
Tedion 25%, WP	1	5.7	13	7.3	5.8
Kelthane 18.5%, WP	2	12.4	14	23.3	
Tedion 25%, WP+DN-111 20%, WP	0.5 0.25	17	12.6	4.7	9.3
Check (unsprayed)	—	21	67	125	

thorough, dilute application of Kelthane would provide good control. One-acre plots were sprayed with air carrier equipment using 1,000 gallons per acre on July 21 and 22. The treatments were evaluated by 100-leaf samples taken from each treatment at weekly intervals. Kelthane 40W at 1½ pounds per 100 gallons of water (equivalent to approximately twice the standard dosage), and Chlorobenzilate® (ethyl 4,4′-dichlorobenzilate) failed to control these mites (table 3). Dimite slowly reduced the population level, but failed to provide adequate control.

Test No. 4—Orondo.—One-half-acre plots were sprayed on August 6 with air carrier equipment, using 1,200 gallons per acre. Samples of 50 leaves were taken from each treatment on each date (table 4). Emulsifiable Kelthane initially reduced the population, but after 11 days, the population had started to increase and the plot was resprayed (table 4). Sulphenone® (*p*-chlorophenyl phenyl sulfone and related sulfones) provided adequate control, but the population was low on this plot prior to spraying. Chlorobenzilate brought about a slow reduction in the population, while ovex failed completely to control the mites.

Test No. 5—Entiat.—A grower in the Entiat area applied two Kelthane sprays during 1959 and had not obtained adequate mite control. Test plots were established in his orchard to determine the effectiveness of Kelthane against these mites. A high population of McDaniel mites was present and damage was evident when the prespray samples were taken.

Single-tree plots replicated three times in a randomized block design were sprayed on July 7 with conventional high pressure equipment and orchard guns at 600 p.s.i. A rate of 1,400 gallons per acre was used. Samples of 25 leaves per replicate were taken at each sampling date (table 5).

Failure to obtain adequate control was general in this test, and the number of mites present had almost doubled on all three Kelthane plots within 2 weeks after application (table 5). Tedion and Tedion plus DN-111 (4,6-dinitro-*o*-cyclohexylphenol *N*,*N*-dicyclohexylamine salt) prevented a mite increase over the period the plots were observed. One possible reason for the general failure to obtain control in this test was the presence of large numbers of the orange, winter forms of the mites. This form is more difficult to control than the green, feeding form.

Discussion.—Applications of Kelthane early in the year failed to prevent a buildup of the mites, and later applications failed to control them. Therefore, timing was not the principal reason for the lack of control. The failure of handgun applications, semiconcentrate applications with air carrier equipment, and thoroughly applied, dilute applications with air carrier equipment rules out the possibility that inadequate coverage was responsible for the control failures. Although the different formulations of Kelthane were not analyzed chemically, material from the same packages gave excellent control in other locations. This is, in effect, a bioassay and indicates that the active material was effective against nonresistant strains of mites.

If the timing, application, and material were not responsible for the lack of control, the failures must have been caused by a change in the susceptibility of the mites to Kelthane. The reports of several growers that they were unable to obtain control of the McDaniel mite with Kelthane, or that more numerous applications were necessary to obtain control tends to substantiate a change in the mite's susceptibility. Although a complete study of related chlorinated hydrocarbon acaricides has not been made, these tests indicate that some tolerance of the mites in these orchards to Dimite, Chlorobenzilate, ovex, and Genite may have been present.

LABORATORY STUDIES.—*Materials and Methods.*—Some evidence was observed in 1958 that mites in one location were resistant to Kelthane. When other reports of control

Table 4.—McDaniel mite control with chlorinated hydrocarbons on apples. Orondo, 1959.

Material	Rate per 100 Gallons	Average Number of Mites per Leaf on Each Sampling Date: Prespray Aug. 5	Aug. 10	Aug. 17	Aug. 20	Aug. 26	Sept. 3
Kelthane 18.5%, EC	2 pt.	11	1.5	4.8			
Chlorobenzilate 25%, WP	1.5 lb.	64	9.6	9.8	3.6	1.4	8.4
Ovex 50%, WP	0.33 lb.	21	20	28			
Sulphenone 50%, WP	2 lb.	8	1.8	1.6	1.2	.1	0

Table 6.—Laboratory studies on the effects of Kelthane sprays on strains of the two-spotted spider mite from different areas of eastern Washington. 1959.

Source of Mites	No. of Replicates	Per Cent Mortality with Different Amounts of Kelthane WP in Pounds per 100 Gallons							LD-50 in Pounds (est.)
		2	1	0.5	0.25	0.125	0.062	0	
Greenhouse strains									
(on peach leaves)	10	—	71	55	51	31	22	—	0.3
(on apple leaves)	10		83	67	47	46	42	—	.15
(on pear leaves)	5	98	96	38	32	26	—	6	.3
Malaga (pears)	5	—	9	84	80	26	—		.3
Rock Island (peach)	10	97	95	64	59	26	—	15	.2
Wenatchee (apples)	10	95	83	63	39	12	—	10	.35
Wenatchee (peach)	10	99	100	98	36	28	—	6	.2
Columbia View (peach)	10	78	86	34	26	22	—	2	.35
Columbia View (peach)	10	99	97	87	42	23	—	6	.25
Quincy (peach)	5	98	94	76	30	18	—	2	.3
Walla Walla (peach)	5	96	92	66	36	14	—	0	.35

difficulties were received in 1959, mites were brought from the orchards and treated on the same day in the laboratory to measure their resistance to Kelthane or other materials. Suspected strains were then tested in this way and a survey was made by testing mites from as many different areas as time permitted.

The sprays were tested at five different dosages. Two quarts of spray at the highest concentration were mixed in a 2,500-ml. separatory funnel. A spreader, X-77, was added with an eye dropper until even wetting of the leaves was obtained. The concentration of the spray was halved successively through five dilutions by draining off a quart of the mixture and replacing this with a quart of water. Each time the spray was diluted by one-half, the wetting efficiency was held at the same level by adding back one-half of the original amount of wetting agent, equivalent to that drained off with the discarded aliquot. The sprays were thoroughly mixed and were constantly stirred with an electric mixer while being used.

Selected leaves were dipped and evenly wetted in the spray mixtures. After the spray dried, discs thirteen-sixteenths of an inch in diameter were cut from the leaves with a cork borer. The leaf discs were placed with the upper side down on slightly larger discs (1 inch in diameter) of white blotting paper. These were put on cellulose-sponge pads 3 inches in diameter and one-half inch thick in 100-mm. petri dishes partially filled with water. Usually, five discs or replicates were placed in each petri dish and each disc was infested with 10 adult mites from selected shoots brought from the orchards. The mites were moved to the discs with a brush made by cementing a single, human eyebrow hair to the end of a wooden handle.

Mites from apple trees were tested on discs from apple leaves and those from peach and pear trees were tested on leaf discs of peach and pear, respectively, to avoid any effects of change in host plants. The petri dishes were left uncovered at room temperatures of about 75° F. and were examined after 3 days. This interval seemed best because it permitted most of the acaricidal effects to be shown before appreciable natural mortality occured. The mites showed less tendency to move into the moisture barrier when kept in subdued light and it is believed that the number lost did not contribute excessively to the experimental error. The LD-50s and estimates of their reliability were calculated according to methods outlined by Bliss (1935).

Results and Discussion.—Results of the tests on the two-spotted spider mite, *Tetranychus telarius* (L.),[6] are given in table 6. The laboratory strain maintained for several years on potted peach seedlings in the greenhouse showed no difference in susceptibility to Kelthane on treated peach and pear leaves. There was a slightly higher mortality of the mites on treated apple leaves but this probably was not significant. Data for the field collections from different areas do not show any marked differences in susceptibility of the mites to Kelthane that would indicate any substantial amount of Kelthane resistance in these strains.

Data on the McDaniel mite are given in table 7. These

[6] Mites identified by Everett C. Burts.

Table 7.—Laboratory studies on the effects of Kelthane sprays on strains of the McDaniel mite from different areas of north central Washington. 1959.

Source of Mites	No. of Replicates	Per Cent Mortality with Different Amounts of Kelthane WP in Pounds per 100 Gallons							LD-50 in Pounds
		2	1	0.5	0.25	0.125	0.062	0	
North Orondo (apples)	10	19	8	6	4	3	—	9	28.0
Methow (apples)	10	97	95	62	38	20	—	3	0.3
Rock Island (apples)	5	98	94	88	70	43	—	8	0.14
South Orondo (apples)	10	53	17	11	4	4	—	2	3.0
Entiat (apples)	5	—	62	42	30	26	16	0	0.64

results show some marked differences in the LD-50s for the different strains. That for the collection from north Orondo of 28 pounds of 18.5% Kelthane wettable powder indicated a 200-fold resistance over the strain from Rock Island and a resistance of over 90-fold as compared with the strain from Methow. Data for the strains from south Orondo and Entiat showed resistance of 17-fold and 5-fold, respectively, as compared with the Rock Island strain. Standard errors of the log concentration-probit regression lines indicate that the most probable value of the LD-50 for the Rock Island mites lies between 0.08 and 0.27 pounds while that for the Methow strain is between 0.2 and 0.4 pounds. These ranges slightly overlap, but show considerable presumptive evidence of some resistance in the Methow strain. Similarly, the analyses showed significant levels of Kelthane resistance in the remaining strains. For the two strains from the Orondo area the lower confidence limits of the LD-50s were much greater than necessary to show resistance.

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Sorghum as a Host of the Southwestern Corn Borer[1]

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ABSTRACT

Infestations of the southwestern corn borer, *Zeadiatraea grandiosella* (Dyar), on sorghum are usually low. Despite this general condition, an investigation was conducted to test the potential of overwintering borers in this crop as a source of infestation in corn. Three types of sorghum (grain, grain-forage and forage) were used. About one-third of the larvae were established on vegetative stages of all varieties but establishment was lower when more mature plants were used. The larval growth rate on sorghum was slightly lower than the rate reported on corn. Overwintering (immaculate) larvae usually entered the plant crowns and girdled the stalk as they do in corn. Some attempted to hibernate in the stem and in side shoots above ground. Most of them above ground died in early winter but those below ground had a low mortality, a relationship that is reported for the insect in corn. More larvae survived the winter in dry than in moist crowns. Survival in crowns was higher in girdled than in nongirdled stalks. Larvae overwintered in sorghum and corn showed about the same period and per cent of moth emergence. Egg production and fertility were a little higher for moths from corn than from sorghum.

Low infestations usually occurring on sorghum apparently result from the relatively low level of attractiveness of this crop to ovipositing moths. Despite the low borer population, this study indicates that sorghum may serve as an important source of overwintered borers to produce infestation in corn. It has not been demonstrated that the borer can be maintained on sorghum alone.

The southwestern corn borer, *Zeadiatraea grandiosella* (Dyar), primarily a pest of corn, was reported infesting sorghum in the United States in 1930 (Todd & Thomas 1930). Infestations have been reported in five other states: Arizona (Davis *et al.* 1933), Arkansas (Rolston 1955), Kansas (Wilbur *et al.* 1942, Wilbur *et al.* 1950), New Mexico (Davis *et al.* 1933), and Oklahoma (Walton & Bieberdorf 1948). The reported incidence of borers in sorghum varies from 4% to almost 100% of the plants infested (Wilbur *et al.* 1942, Rolston 1955). Rolston noted that when corn was dried by drought to the extent that it was unattractive for oviposition, sorghum remained green and was heavily infested with second generation larvae. While the reported damage to sorghum by this borer is seldom important, the potential of this crop to serve as a reservoir of overwintered borers and thus increase the damage to corn in the succeeding season has received little attention. Little information is recorded on the establishment, development and survival of this species in sorghum. To obtain this knowledge a field experiment was conducted at Stillwater, Oklahoma during 1956–57, with special emphasis given to a study of overwintering.

METHODS AND MATERIALS.—Five sorghum varieties (two grain type, two forage type, and a grain-forage type) commonly grown in Oklahoma were used. These varieties with references to description are as follows: Redlan (Davies & Sieglinger 1952), Darset (Davies & Sieglinger 1953), Hegari 750 and African Millet (Finnell 1929), and Sugar Drip (Sieglinger *et al.* 1949).

The five varieties were planted May 25, 1956 in unreplicated, 4-row blocks, with rows 42 inches apart and 150 feet long. When plants were about 4 inches tall, they were thinned to a spacing of 4 to 6 inches. An attempt was made to simulate the cultural practices common under usual field conditions, but because of drought the planting was irrigated three times during the growing season. Irrigation was used only after plants were wilting severely and a serious threat of loss of plants was evident.

Since the natural infestation in sorghum is usually very low, and because a higher and controlled level of

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Table 1.—Record of manual infestations of sorghum, 1956.

PERIOD WHEN EGGS WERE PLACED ON PLANTS	NO. OF PLANTS INFESTED PER VARIETY	STAGE OF PLANT DEVELOPMENT
July 6–11	120	Vegetative
July 22–31	120	Boot to dough
Aug. 10–13	120	Late dough
Sept. 2–12	80	Mature

infestation was needed to make the desired observations, plants were manually infested. The sorghum was infested in four periods on plants of different stages of development by using eggs deposited on waxed paper by moths in laboratory cages (table 1). When egg fertility became evident (Hensley & Arbuthnot 1957) the paper was cut into small fragments, each containing five fertile eggs. Just prior to hatching of the eggs, a paper fragment was pinned to a leaf sheath or stalk of each plant. During the early part of the season eggs were attached to the leaf sheath near the base of the stalk, but later, when the plants approached maturity and leaf tissue on the lower portion of the stalk dried, the eggs were placed on the stalk. The number of eggs hatched on each plant was recorded 48 to 72 hours after they were placed on the plants.

Girdled plants of each four infestations were used to determine survival of the overwintering brood through moth emergence. On May 22, 1957 cages were placed over individual sorghum stubs to prevent escape of the moths upon emergence. These cages were made from 6-inch sections of 1½-inch diameter glass tubing with the upper end covered with nylon mesh. Cages were inspected for moth emergence twice daily, during the early morning and again in the evening. Records were kept on the origin of individual moths.

Fifty girdled corn plants in a nearby field, were treated in the same manner as the sorghum to obtain comparative data between the two crops. The corn and sorghum were in a similar soil type and were approximately 200 feet apart.

Each of the moths obtained from field cages was isolated until its sex was determined. Pairs were placed in 6-inch long, 1½-inch diameter glass cylinders which were closed at both ends with nylon mesh, and lined with waxed paper. Dead moths and the waxed paper linings were removed daily, and the numbers of fertile and infertile eggs were determined for each pair.

Data concerning the hatching of fertile eggs were not recorded because fluctuations of temperature and humidity in storage may have affected development; however, it was observed that many of the fertile eggs hatched.

RESULTS AND DISCUSSION.—*Survival and Development of Summer-Form Borers.*—Survival evaluations of summer-form borers (Davis *et al.* 1933) were obtained from sorghum at periods of 1 to 40 days after egg deposition. Dissection of five plants per variety at 1 day and 6 days after hatch, showed approximately 30% became established. At 38 to 40 days after egg deposition, borer survival was 18.5 % (table 2). An examination of plants in late October showed 22.2% of the hatched eggs produced immaculate larvae or moths (table 3).

Table 2.—Survival and development of borers 38 to 40 days after egg deposition on vegetative stage sorghum.[a]

CATEGORY	VARIETY OF SORGHUM					ALL VARIETIES
	Redlan	Darset	Hegari	African Millet	Sugar Drip	
No. of hatched eggs	123	122	130	134	136	645
No. of forms recovered	21	22	15	25	36	119
Per cent survival	17.1	18.0	11.5	18.7	26.5	18.5
Per cent in stages:						
Instar IV	0	9.0	13.4	0	5.6	5.0
Instar V	14.2	31.8	20.0	0	19.4	16.8
Instar VI	54.2	36.4	60.0	36.0	47.2	45.4
Pupal	33.3	4.5	6.7	60.0	25.0	28.6
Pupal cases	0	18.2	0	4.0	0	4.2

[a] Based on 30 plants per variety.

Table 3.—Survival of borers to moth emergence or to the immaculate larval stage in sorghum infested in the vegetative stage.[a]

BORERS	VARIETY OF SORGHUM					ALL VARIETIES
	Redlan	Darset	Hegari	African Millet	Sugar Drip	
No. of hatched eggs	370	396	379	419	345	1,909
No. recovered	77	69	94	93	88	423
Per cent:						
Survival	20.8	17.4	24.8	22.7	25.5	22.2
Emerged as moths[b]	77.9	65.2	70.2	69.5	79.5	72.6
Attained immaculate stage[c]	22.1	34.8	29.8	30.5	20.5	27.4

[a] Based on 90 plants per variety, examined late October.
[b] A pupal case or gate was recorded as an emerged moth.
[c] Each crown of a girdled stalk was assumed to contain one living immaculate larva.

Apparently the greatest mortality of borers occurs soon after hatching, since less than one-third of those that hatched became established. During the remainder of the developmental period mortality increased only about 15%. Hensley (1955) reported that approximately one-third of the larvae on corn survived through the first instar and that little mortality occurred during later larval instars or the pupal stage. Mortality on sorghum appears to be similar to that on corn.

Mortality appears to be greater for borers attempting to establish on older, mature plants. Survival to larval maturity or moth emergence on sorghum, infested when plants were mature, was only 2.6% of hatched eggs. In such plants, borers were found only in the greener portions, indicating these tissues were favorable for survival or had attracted migrating larvae. Hensley (1955) found a similarly low survival (4%) of second generation borers on drought damaged corn plants.

Survival on sorghum was found by Rolston (1955) in laboratory, insectary, and field tests to range from approximately 50% to 100% of the survival on corn. Survival of first generation borers on normally growing corn was estimated by Hensley (1955) in Oklahoma to be 32.9%.

Varietal effects may be a factor in survival of summer-form borers on sorghum. Borers on Sugar Drip showed higher survival than those on other varieties at both 38 to 40 days after oviposition and in late October.

Limited information was obtained on the rate of de-

Table 4.—Location of borers in three types of sorghums infested in the vegetative stage.

Borers	Summer-Form[a] Borers		Pupal Cases[b]			Immaculate Larvae[b]		
	Grain	Forage	Grain	Grain-Forage	Forage	Grain	Grain-Forage	Forage
Total number	43	61	105	66	136	50	28	47
Per cent:								
Below ground	7.0	14.8	9.5	9.1	11.8	56.0	78.9	93.6
In 1–7th internodes	56.1	68.8	31.4	45.5	48.5	22.0	14.3	6.4
Above 7th internode	16.3	16.4	36.2	42.3	38.2	22.0	7.0	0.0
In peduncle	20.6	0	22.8	3.0	1.5	0	0	0

[a] Based on dissection of 30 plants each of Redlan and Darset (grain) and African Millet and Sugar Drip (forage), 38 to 40 days after egg deposition.
[b] Based on dissection of 90 plants each of Redlan and Darset (grain), Hegari (grain-forage), and African Millet and Sugar Drip (forage), in late October.

velopment of borers in sorghum infested during the boot to dough stage. For this purpose five plants per variety were dissected at intervals of 4 to 6 days, beginning at egg hatch.

On Redlan the first instar larvae were found 6 days after egg deposition; second instar, 6 to 10 days; third instar, 10 to 19 days; fourth instar, 14 to 25 days; fifth instar, 14 to 25 days; and, sixth instar, 25 to 36 days. Survival in Darset, African Millet and Sugar Drip was low but the larvae that survived developed at a rate similar to those in Redlan. Larvae in Hegari, however, developed at a slower rate and none beyond the fourth instar was found.

In a similar study on corn, Hensley (1955) found the rate of borer development to be essentially equal to that found in Redlan. Rolston (1955), in laboratory studies, found the average duration of the larval stage to be 22 days on corn and 29 days on sorghum.

Location of Summer-Form Borers.—Data on the location of borers in sorghum plants are given in table 4. In a dissection made 38 to 40 days after egg deposition they were found in plant crowns, in internodes 1 to 9, and in the peduncle or whorl; but 63.1% to 83.6% were in the crown and internodes 1 to 7, which constitute the lower half of the plant. There was a difference in the location of borers between the grain and forage type sorghums. In the grain type, 20.6% were in the peduncle or whorl, while in the forage type, no borers were in these parts. Samples taken in late October, showed 22.8% of the pupal cases were in the peduncle of the grain type as contrasted with only 3.0% and 1.5% in this part of the grain-forage and forage types, respectively. The grain varieties had about one-half of all borer forms (immaculate larvae and pupal cases) above the seventh internode while the other varieties contained approximately one-third of the population in this region.

Data concerning the location of larvae in plants infested in the boot or dough stage are few but certain trends are evident. Larvae of the first three instars were usually found in the partially enclosed head or boot of the plant. Where heads were exposed, the larvae were under leaf sheaths, occasionally as low as the third internode and most of them were found in succulent plant tissues. Only fourth or later instar larvae tunneled into the stalks.

Further evidence of the association of borers with succulent tissues was obtained in October from dissection of plants infested in the vegetative stage (90 plants per variety). In this group of immature borers, 14.4% were contained in green suckers, growing on the parent stalks, although no eggs had been placed on them. Toward the end of the growing season, in a second group of plants, larvae were found only in the greener portions of the plants. In a later dissection, all borers found above the ground were in green shoots.

These effects of stage of borer development and condition of plant tissue on the location of borers in sorghum are similar to those reported by Hensley (1955) and Hensley & Arbuthnot (1957) on corn. With few exceptions they found that early instar larvae on whorl-stage corn fed in the whorls, but as whorls opened and their tissues became tougher the young larvae were attracted to more succulent tissues within leaf sheaths or on secondary shoots.

Location of Overwintering Larvae.—From a total of 1,900 plants infested throughout the summer and left undisturbed in the field until October 27, about 10% were girdled by larvae in preparation for overwintering. The typical location of overwintering (immaculate) larvae in sorghum was found to be the crown, as reported for corn; however, some deviations were noted (table 5).

Table 5.—Location of overwintering (immaculate) larvae in late October in five varieties of sorghum infested in the vegetative stage.

Larvae	Redlan	Darset	Hegari	African Millet	Sugar Drip	All Varieties
Total number[a]	22	28	28	29	18	125
Per cent:						
Below ground	59.1	53.5	78.6	93.1	94.4	75.2
Above ground	40.9	46.5	21.4	6.9	5.6	24.8

[a] It was assumed that each girdled plant contained one larva.

There was a relation between plant height and the location of borers; the percentage of borers found below ground was greater in the taller, forage type varieties. Almost one-half of the immaculate larvae in the shorter grain type varieties were above the ground and many of these were in secondary shoots, where they often prepared winter quarters. In several cases, where larvae attempted to hibernate in side shoots or in the upper portion of the main stalk, girdling occurred above the site of the borers, and even as high as the peduncle.

Mortality of Overwintering Larvae.—One of the principal objectives of this study was to determine the overwintering survival of the southwestern corn borer in

sorghum. Factors investigated in relation to larval mortality included sorghum varieties, location of larvae, girdling of stalks, moisture content of plant crowns, and comparative mortality in sorghum and corn.

No difference was observed between varieties relative to overwintering borer mortality, except in the case of Hegari which is discussed under spring moth activity.

No living immaculate larvae were found above ground level on December 29 but mortality of those below ground was only 11.8% (table 6).

Table 6.—Mortality of overwintering larvae above and below ground level in sorghum.

LARVAE	DATE OF DISSECTION			
	Nov. 3	Dec. 1	Dec. 29	Jan. 26
Total number	32	26	19	10
Per cent:				
Above ground	18.8	23.1	10.5	20.0
Below ground	81.2	76.9	89.5	80.0
Mortality above ground	0	66.6	100.0	100.0
Mortality below ground	3.8	10.0	11.8	37.5
Total mortality	3.1	23.1	21.1	50.0

Evidently girdling of the plant by this borer increases winter survival in plant crowns. Six dissections made at approximately monthly intervals from November 3 to March 23 showed survival to be consistently higher in girdled than in nongirdled plants (table 7).

Table 7.—Mortality of overwintering larvae below ground in girdled and nongirdled sorghum plants.

LARVAE	DATE OF DISSECTION					
	Nov. 3	Dec. 1	Dec. 29	Jan. 26	Feb. 23	Mar. 23
Number:						
In girdled plants	15	12	14	4	12	8
In nongirdled plants	11	8	3	4	3	4
Per cent mortality:						
In girdled plants	0	0	7.1	0	0	25.0
In nongirdled plants	9.1	25.0	33.3	75.0	33.3	75.0
In all plants	3.9	10.0	11.8	37.5	6.7	41.7

Excess moisture in plant crowns appeared to be an important factor in mortality of overwintering larvae (table 8). In dissections made on five dates from December 1 to March 23, mortality in moist plant crowns (containing free water) ranged from 20% to 100%, and averaged 48.0%, but in dry plant crowns it ranged from 0% to 7.1% and averaged 2.1%. Of the 27 larvae included in the study, 92.3% of the dead were in moist crowns. Excess moisture in plant crowns may occasionally result in drowning of larvae. However, it is believed that the chief injury resulting from moisture occurs when the larvae, in order to escape the moisture, move upward in the plant and, thus, become exposed to unfavorable effects of fluctuating temperatures. Some of the dead larvae removed from standing plants which contained water in the crowns were found in the first few internodes above soil level. Walton & Bieberdorf (1948) observed that where free water occupied the hibernation tunnels in crowns of corn, frequently the larvae were found, dead or alive, in the plant a few inches above the soil.

Table 8.—Mortality of overwintering larvae in dry and moist plant crowns.

LARVAE	DATE OF DISSECTION				
	Dec. 1	Dec. 29	Jan. 26	Feb. 23	March 23
Number:					
In dry plants	10	14	5	12	6
In moist plants	10	3	3	3	6
Per cent mortality:					
In dry plants	0	7.1	0	0	0
In moist plants	20.0	33.3	100.0	33.3	83.3

A total of 108 girdled sorghum plants were left in the field undisturbed until spring moth emergence was complete (table 9). Records of moths trapped from these plants showed 67.6% of the overwintering forms survived to emergence. A parallel study of girdled corn plants showed 62.0% survival. These results differ from the findings in Kansas (Wilbur *et al.* 1950) where, in January, only 35% of the larvae were alive in sorghum but 70% survived in corn. No other direct comparisons of survival on the two crops have been noted in literature.

Table 9.—Fate of overwintering borers in girdled plants of sorghum and corn.

BORERS	SORGHUM	CORN
Number of plants examined	108	50
Per cent mortality:		
Missing larvae[a]	15.7	16.0
Dead larvae	3.7	4.0
Dead pupae	11.1	18.0
Moths (dead in stubble)	1.8	0
Per cent moth emergence	67.6	62.0

[a] It was assumed that each girdled plant initially contained one immaculate larva and when none was found it was assumed that it died.

Spring Moth Activity.—Spring moth emergence from sorghum began about May 22 and ended June 17. On corn it began about May 25 and ended June 13. Moths emerged from 68% of the 108 girdled sorghum plants and 62% of the girdled corn plants (table 9). In girdled plants, survival through spring moth emergence ranged from 60% to 72% for all sorghum varieties except Hegari where the percentage was only 45. Crowns of Hegari were generally deteriorated, thereby further exposing the overwintering borers to weather conditions. The time when plants were girdled did not appear to affect survival or time of moth emergence.

Of 72 moths emerged from sorghum and 27 from corn, 48.6% and 48.1%, respectively, were females. During the first third of the emergence period males were in the majority, with the first female appearing 4 to 5 days after emergence began. In the second third of the period the sexes were about equal in number but females predominated later. Davis *et al.* (1933) stated that emergence of both sexes starts at about the same time and that

the ratio remains fairly constant throughout the season, the females being more numerous.

Fecundity, Fertility and Longevity of Moths.—Data were obtained on the reproductive capacity of moths from borers overwintered on sorghum and corn. Thirty-two of 35 females that emerged from sorghum, and 7 of 8 from corn, deposited eggs (table 10). Moths from sorghum deposited both the largest and smallest number of eggs per individual, showing a range of over 500 eggs. The average numbers of eggs and per cent of eggs fertile were a little higher for the moths from corn than from sorghum.

The average length of life of moths from sorghum was 5.4 days for 37 males and 5.7 days for 34 females. Corresponding records for moths from corn were 5.8 days for 4 males and 5.2 days for 8 females. This longevity is similar to that reported by Rolston (1955).

Table 10.—Fertility and fecundity of female moths from borers overwintered in sorghum and corn.

Performance	Sorghum	Corn
Number of moths:		
Total observed	35	8
Laying only infertile eggs	7	0
Laying both fertile and infertile eggs	25	7
Number of eggs per moth:		
All moths	315.7	377.5
Moths laying both fertile and infertile eggs	330.5	377.5
Per cent of eggs fertile	87.6	93.0
Range in fecundity and fertility:		
Number of eggs per moth	76–580	247–520
Per cent of eggs fertile	59–98	83–99

Occasionally egg deposition occurred during the first night after moths emerged from sorghum, however, all these eggs were infertile. Fertilized moths deposited about 40% of their eggs the second night after emergence (table 11). Deposition then declined consistently until only about 1% of the total eggs were deposited on the sixth night. Infertile moths laid eggs more evenly over a longer period of time, sometimes ovipositing as late as the eighth night after emergence.

Table 11.—Egg production and fertility of 22 moths from borers overwintered in sorghum.

Day After Emergence	No. of Moths Ovipositing	Total Eggs	Per Cent of Total Eggs Deposited	Per Cent of Eggs Fertile
First	6	45	0.6	0
Second	17	2,976	39.5	91.5
Third	19	2,208	29.3	96.1
Fourth	20	1,646	21.8	89.4
Fifth	17	560	7.4	71.2
Sixth	6	84	1.1	53.6
Seventh	2	15	0.2	26.7
Eighth	1	1	0	0

The highest percentage of fertility (96) was found in eggs deposited the third night after emergence. Fertility of eggs deposited on the second and fourth nights approached that of the third night with about 91% and 89%, respectively. Occasionally a moth would deposit eggs freely the first or second night, fail to oviposit for one night, and resume laying later.

Sorghum as a Reservoir of Infestation for Corn.—Under the conditions of this study the development and survival of *Z. grandiosella* on the typical sorghum varieties were similar to its performance on corn. The average time required for summer-form larvae to reach maturity on sorghum was somewhat longer than that reported by other workers for this species on corn. The percentages of winter survival of larvae and emergence of moths on sorghum were slightly higher than on corn. Moth emergence, fecundity, and egg fertility were essentially equal for the two crops.

Natural borer infestations on sorghum are generally much lower than those occurring on corn of similar vigor and succulence (Davis *et al.* 1933, Walton & Bieberdorf 1948, Rolston 1955). The small amount of damage usually sustained by sorghum in the field, despite the demonstration of host suitability when plants were manually infested, apparently results from the relatively low level of attractiveness of this crop to ovipositing moths. This is supported by Wilbur *et al.* (1943) who reported the highest average infestations in fields of sorghum to be 7% but volunteer sorghum plants in corn fields had infestations as high as 54%. Furthermore, volunteer corn plants in sorghum fields and sorghum plants adjacent to them were infested. Under conditions of drought, when corn has deteriorated to the extent that it is no longer attractive to moths, sorghum may retain sufficient vigor and succulence to be attractive for oviposition and suitable for borer development.

Rolston (1955) reported that in Arkansas, in an experiment on late-seeded plots of growing plants of the same age, more than 10 times as many eggs were laid on corn as on sorghum. However, at about the same time early-seeded, drought-stricken corn was unattractive for oviposition but a field of sorghum and a field of late, irrigated corn were heavily infested.

Prior to the appearance of widespread heavy infestations of the southwestern corn borer in Oklahoma, considerable acreages of corn were planted during June. In the presence of high populations, this corn was attractive to moths in July and August and infestations involving essentially all of the plants resulted (Walton & Bieberdorf 1948). Collections made at this experiment station during several years showed late corn contained a high incidence of hibernating borers. Spring emergence of moths from late plantings are believed to have contributed to first generation infestations in nearby fields of early corn.

Because of the severe damage sustained to late corn this type of crop became progressively less popular and was largely replaced by sorghum. In addition, the proportion of total corn acreage to sorghum acreage has markedly decreased, brought about by both borer injury and recognition by farmers of the greater adaptability of sorghums to climatic and soil conditions of the state. In 1940 the corn to sorghum acreage ratio was 44:56 but by 1958 it was 19:81 (Oklahoma Crop and Livestock Reporting Service, 1947, 1959).

Because of the high proportion of sorghum to corn and the relatively high borer survival on sorghums, and despite the low population usually present, this crop appears to serve as an important source of overwintered borers to infest corn. This dependence on sorghums is particularly important following a year when corn prematurely dies from drought. Under such conditions, the local sources of overwintered borers are largely limited to sorghums which are more drought resistant and able to approach or reach maturity.

In contrast to the favorable conditions provided by sorghum for overwintering, this crop is not a favorable host for the first brood of borers. Sorghum is usually planted later, germinates more slowly and has slower seedling growth than corn. For these reasons the plants are smaller or even not available when moths from the overwintered brood are seeking oviposition sites. In the absence of corn, the first brood is, therefore, largely eliminated and later broods are greatly reduced.

While it has not been demonstrated that the borer can be maintained on sorghum alone, this study demonstrates that where both crops are grown in the same community the borer problem on corn will be increased.

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Germination of Small Legume Seeds After Fumigation with Methyl Bromide and Hydrocyanic Acid[1]

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ABSTRACTS

Five species of small legume seeds (Ranger alfalfa, *Medicago sativa;* alsike clover, *Trifolium hybridum;* ladino clover, *Trifolium repens;* Kenland red clover, *Trifolium pratense;* and Viking birdsfoot trefoil, *Lotus corniculatus*) with a graded moisture range approaching 6%, 9%, and 12% were fumigated with methyl bromide and hydrocyanic acid in 100-cubic-foot gas-tight chambers. Exposure periods, dosages of fumigant applied, and temperatures during fumigation were varied. One-half of the seed samples received one fumigation; the others were fumigated twice.

Under the conditions of these studies, percentages of germination of alsike clover seeds were reduced by exposure to hydrocyanic acid; seeds of the other species demonstrated tolerance to this fumigant. Germination of Viking birdsfoot trefoil seeds was not impaired by fumigation with methyl bromide, but reductions in percentages of germination were observed in tests with seeds of Ranger alfalfa, alsike clover, ladino clover, and Kenland red clover after fumigation with methyl bromide under some conditions.

Differences between species in susceptibility of seeds to fumigation with methyl bromide and hydrocyanic acid was recognized by variations in percentages of germination ranging from complete tolerance of some species to economic reductions in germination of others. Where reductions in germination occurred after fumigation of seeds, variables probably of most importance in contributing to injury of seeds during fumigation were length of exposure to fumigant, dosages applied, moisture content of seeds, and repeat fumigations.

Little attention was given to the effect of fumigation on germination of small legume seeds prior to initiation of the Khapra Beetle Eradication Program in California as control measures against storage pests in such seeds were seldom necessary. Complete fumigations required to exterminate the khapra beetle, *Trogoderma granarium* Everts, wherever infestations were found lent emphasis to the need for information on susceptibility to fumigants of all kinds of seeds in storage.

Results presented in a previous report from this lab-

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oratory indicated that germination was reduced in 3 of the 20 varieties of small legume seeds tested after fumigation with 4 pounds of methyl bromide per thousand cubic feet for 12 hours at 70° F. (Lindgren *et al.* 1955). Reduction in germination of small legume seeds was not evident after fumigation with one-half pound of hydrocyanic acid per thousand cubic feet for 12 hours at 70° F. Later Cobb (1958) reported on studies in the Federal-State Seed Laboratory of the California Department of Agriculture in which an effort was made to evaluate the influence of several variables on the germination of seeds fumigated with methyl bromide for control of the khapra beetle. Results from his tests with ladino clover, white clover, and purple vetch indicated reductions in germination which could be correlated with increases in moisture content of seeds, especially in tests with seeds that were fumigated twice with methyl bromide.

The possibility of causing a reduction or failure in germination of small legume seeds so long as it is necessary to use heavy dosages of fumigants and long exposures to eradicate the khapra beetle was sufficient reason to further evaluate several variables which might influence the germination of seeds exposed to methyl bromide or hydrocyanic acid. It is the purpose of this paper to summarize results from germination tests with seeds of five species of small-seeded legumes which were exposed to these two fumigants under various controlled conditions.

MATERIALS AND METHODS.—*Preparation of Seed Samples.*—The initial moisture content of seeds of the legumes selected for this study were: Ranger alfalfa (*Medicago sativa*), 5.5%; alsike clover (*Trifolium hybridum*), 9.1%; ladino clover (*Trifolium repens*), 6.1%; Kenland red clover (*Trifolium pratense*), 6.6%; and Viking birdsfoot trefoil (*Lotus corniculatus*), 7.5%. Moisture content was determined by drying 2-gram samples of whole seeds in a mechanical-convection oven for 1 hour at 130° ± 3° C. and calculating the per cent loss in weight. Four samples were dried for each moisture determination.

Samples of seeds approaching the desired graded moisture range of 6%, 9% and 12% were obtained by careful adjustment of moisture and conditioning. Seeds were dried in narrow wire-screen containers placed in a forced-air oven maintained at 125° to 130° F. This temperature did not affect the germination of those seeds that required drying. The drying period ranged up to, but never longer than 3 hours at any one time. The moisture content of seeds before drying and the desired moisture level indicated the length of time seeds were to be held in the air-oven. Water was added to increase the moisture content as needed. After drying or addition of water, seeds were conditioned in airtight polyethylene bags kept inside sealed metal containers for 7 days at 50° F. Conditioned seeds were blended and moisture tests were made to determine the need for additional moisture adjustments.

When the moisture content approached the desired level (see tables for actual moisture of seeds used) samples were placed in small rough-woven cotton bags. Seeds were removed from these bags only for germination tests. Each cloth sample-bag of seeds was placed in an airtight polyethylene bag to prevent moisture changes. Prepared samples awaiting fumigation were held in storage at 50° F.

Fumigation of Seeds.—Fumigation experiments were conducted in 100-cubic-foot gastight chambers with continuous circulation of air within the fumatorium. Temperatures were thermostatically controlled at 50°, 70°, and 90° F. ± 1° F., but no attempt was made to control humidity. Fumigations were replicated. Test samples were held overnight at the desired fumigation temperature before removing polyethylene bags. Seeds of all three moisture levels were placed in wire baskets inside the fumigation chambers to be fumigated together. The wire baskets permitted thorough circulation of gases.

Methyl bromide was applied at two dosage levels, one high and one low, throughout the study. Dosages were selected according to exposure period and temperatures during fumigation. The exposure periods, temperatures during fumigation, and dosages of methyl bromide applied are given in table 1. Concentrations of fumigant were not determined for each dosage or exposure interval, but typical curves for the fall in concentration of methyl bromide were given previously by Strong & Lindgren (1959). All seeds received an initial fumigation; one-half of the samples were fumigated a second time 21 days after the initial exposure to test the effect of repeat fumigation. Seeds fumigated with methyl bromide were aerated for at least 24 hours after fumigation before use in germination tests. Seeds to be fumigated twice were aerated for 24 to 48 hours, then repackaged in airtight polyethylene bags and held in storage at 50° F. until ready for use in the second fumigation.

Seed samples were exposed to one and two fumigations with hydrocyanic acid at 2 pounds per thousand cubic feet for 72 hours at 90° F. Only alsike clover was fumi-

Table 1.—Exposure periods, temperatures, and dosages of hydrocyanic acid and methyl bromide used for the fumigation of small legume seeds.

Fumigant	Exposure (hours)	Temperature (°F.)	Dosage (lb./1,000 cu. ft.) Low	High
Hydrocyanic acid	72	90	1.0	2.0
		70	1.0	2.5
		50	1.0	3.0
Methyl bromide	72	90	1.0	3.0
		70	1.5	3.0
		50	2.0	4.0
	48	90	1.5	4.0
		70	2.0	4.0
		50	2.5	5.0
	24	90	1.5	5.0
		70	2.0	5.0
		50	2.5	5.0
	8	90	2.5	5.0
		70	3.0	6.0
		50	3.0	6.0
	2	90	3.0	6.0
		70	3.0	6.0
		50	3.0	6.0

gated with hydrocyanic acid under all of the conditions listed in table 1. After fumigation, seeds were aerated for at least 48 hours before use in germination tests. Seeds to be refumigated were aerated for 48 hours, repackaged in airtight polyethylene bags, and held in storage at 50° F. for use in the second fumigation with hydrocyanic acid.

Germination Tests and Assessment of Results.—Some of the germination tests with alsike clover fumigated with hydrocyanic acid were made with seeds planted in fine vermiculite and held at 68° F. In all other tests, seeds of small legumes were germinated at 68° F. between blotters within 10 days following fumigation. Germination tests and seedling interpretations were made in accordance with regular methods of Official Seed Analysts (Assoc. Off. Seed Analysts 1954) except seeds with mechanical injury were not planted and 100 seeds were used per test for each treatment replicate. Unfumigated seeds served as control standards for comparison. Only normal seedlings (hard seeds excluded) were counted to determine the percentage germination, the criterion used for comparison of results. Seedling interpretations were based on descriptions and illustrations given in the U. S. Department of Agriculture, Agriculture Handbook No. 30 (PMA and Bur. of Plant Industry, Soils and Agricultural Engineering 1952).

RESULTS AND DISCUSSION.—As mentioned previously, the purpose of this study was to evaluate the influence of several variables on the germination of small legume seeds exposed to methyl bromide and hydrocyanic acid fumigation. Variables considered were as follows: (1) species susceptibility to methyl bromide and hydrocyanic acid, (2) moisture content of seeds, (3) temperatures during fumigation, (4) dosages of fumigant, (5) periods of exposure to fumigant, and (6) repeat fumigations. More than 900 germination tests of 100 seeds per test were made during the study, but only a summary of data essential to the understanding and discussion of variables investigated are presented in this report.

Germination in control samples of Ranger alfalfa, ladino clover, and Kenland red clover was good, consistently ranging around 90% in all tests. Lower percentages of germination were obtained in tests with control samples of Viking birdsfoot trefoil. Germinations in control samples of alsike clover were generally low and inconsistent between tests, sometimes fluctuating as much as 20% between samples of the same moisture content. There was, however, no indication that handling of seeds to obtain the desired graded moisture levels was responsible for this condition.

Differences between species in their response to fumigants were not in the same order as observed in results from germination tests with unfumigated seeds. For this reason, differences between species in susceptibility to methyl bromide and hydrocyanic acid fumigation should be noted carefully when considering the summary of results from germination tests with seeds exposed to these two fumigants.

Results from germination tests made with small legume seeds after fumigation with methyl bromide are summarized in table 2 as the mean variations in the percentages of germination of fumigated seeds from unfumigated controls. Although tests were made with seeds

Table 2.—Variations[a] in the percentage germination of fumigated from unfumigated small legume seeds after one and two fumigations with methyl bromide for 72, 48, 24, or 8 hours at three temperatures and varying dosages.

Kind of Seeds	No. of Fumigations	Moisture Content of Seeds (%)	Fumigation Temperatures and Dosages[b] 90° F. Low	90° F. High	70° F. Low	70° F. High	50° F. Low	50° F. High
A. Exposure of 72 hours								
Ranger alfalfa	1	5.8	+ 1	− 5	− 1	− 6	+ 1	− 3
		8.9	− 6	−10	−11	−13	− 5	− 6
		12.0	− 2	−10	− 7	−15	− 4	− 5
	2	5.8	− 4	− 8	− 8	−12	− 7	− 8
		8.9	− 9	−14	− 9	−16	− 9	− 8
		12.0	−11	−12	−12	−13	− 9	−15
Alsike clover	1	6.2	− 1	−15	0	+ 1	0	− 3
		9.1	−10	−17	− 4	− 9	− 9	− 7
		12.2	− 4	−11	− 8	− 6	− 2	−12
	2	6.2	−10	− 6	−13	−14	− 7	−12
		9.1	−11	− 8	−14	− 9	−20	−12
		12.2	− 9	− 9	−15	−18	−16	−13
Ladino clover	1	6.1	+ 3	− 6	− 5	−10	+ 2	+ 7
		9.3	− 1	−21	−15	−18	− 1	0
		11.7	− 4	−20	−20	−22	+ 3	− 4
	2	6.1	−15	−20	−13	−17	−10	− 7
		9.3	−12	−21	−26	−20	−14	−21
		11.7	−12	−19	−18	−18	−14	−27
Kenland red clover	1	5.9	− 1	−19	−11	−14	− 3	− 5
		8.8	− 5	−22	−13	−17	− 4	−10
		11.8	− 6	−13	−17	−18	− 6	−17
	2	5.9	−20	−19	−13	−15	−10	− 7
		8.8	−14	−20	−12	−20	−15	−21
		11.8	−15	−23	−25	−24	−11	−18
Viking birdsfoot trefoil	1	5.8	+ 5	+ 2	+ 1	− 3	+14	+ 8
		9.1	− 6	+ 3	+ 3	− 5	+ 9	+ 5
		11.7	− 2	+10	+ 4	+ 4	+10	+ 5
	2	5.8	+ 4	+ 6	+ 1	0	− 6	− 2
		9.1	− 8	− 2	+ 8	+ 4	− 5	− 8
		11.7	− 2	− 5	− 5	+ 1	− 8	− 2
B. Exposure of 48 hours								
Ranger alfalfa	1	5.8	− 3	−10	+ 1	− 8	− 5	− 4
		8.9	− 6	− 8	− 4	− 8	− 1	− 3
		12.0	+ 1	−15	− 1	−14	− 5	−10
	2	5.8	− 4	− 6	−11	−12	− 3	− 5
		8.9	− 3	− 3	−10	−17	−10	−18
		12.0	− 9	−12	− 9	−15	−14	−17
Alsike clover	1	6.2	− 9	−11	− 3	− 9	+ 1	0
		9.1	+ 5	−10	+ 2	− 3	− 2	− 3
		12.2	− 7	−13	− 4	− 1	+ 4	− 2
	2	6.2	+ 1	+ 1	−13	−12	− 6	− 6
		9.1	+ 5	−24	− 8	−16	+ 1	−16
		12.2	+ 5	−28	−12	−21	− 6	−11
Ladino clover	1	6.1	− 4	− 9	+ 3	− 3	+ 8	− 5
		9.3	+ 2	−10	− 3	+ 4	+ 4	+ 6
		11.7	− 2	−15	− 7	−14	+ 4	− 9
	2	6.1	+ 6	− 8	+ 4	− 5	+ 1	− 2
		9.3	− 6	−13	+ 1	−15	− 4	− 6
		11.7	− 3	−21	− 6	−18	−10	−23
Kenland red clover	1	5.9	− 4	− 9	− 6	− 7	− 2	− 3
		8.8	− 7	−22	− 6	−10	− 2	−11
		11.8	−10	−22	− 1	−10	+ 3	− 9
	2	5.9	0	−18	− 5	−13	−10	−12
		8.8	−10	−21	− 6	−13	−12	−13
		11.8	−14	−18	− 7	−21	−13	−27
C. Exposure of 24 hours								
Ranger alfalfa	1	5.8	− 3	− 4	− 3	− 3	+ 5	− 2
		8.9	− 4	− 2	+ 2	− 3	+ 2	+ 5
		12.0	+ 4	− 4	+ 1	− 3	+ 1	+ 1
	2	5.8	− 2	− 1	+ 1	− 3	0	− 1
		8.9	− 1	− 5	− 1	− 4	− 2	− 5
		12.0	− 4	− 5	+ 3	− 3	− 2	− 1
Alsike clover	1	6.2	+ 1	−11	− 2	− 1	+ 1	− 5
		9.1	− 7	−10	− 4	−10	+ 8	0
		12.2	− 5	−15	− 2	− 6	+10	+ 5
	2	6.2	− 4	−10	+ 8	+ 3	− 4	+ 2
		9.1	− 7	− 6	− 1	− 2	+ 3	+ 1
		12.2	+ 9	+ 3	+ 1	− 9	+ 5	− 3

Table 2 continued from previous page.

Kind of Seeds	No. of Fumigations	Moisture Content of Seeds (%)	Fumigation Temperatures and Dosages[b] 90° F. Low	90° F. High	70° F. Low	70° F. High	50° F. Low	50° F. High
Ladino clover	1	6.1	− 1	− 9	− 3	+ 2	− 3	− 1
		9.3	0	− 4	+ 2	+ 4	− 3	− 4
		11.7	− 2	−13	− 3	− 7	− 1	0
	2	6.1	0	−13	− 2	− 4	− 1	− 2
		9.3	+ 1	− 7	− 1	− 6	0	− 3
		11.7	+ 3	− 2	+ 6	−11	− 4	+ 2
Kenland red clover	1	5.9	+ 4	− 4	+ 1	− 5	− 3	− 1
		8.8	+ 2	− 2	+ 2	− 4	+ 4	− 2
		11.8	− 1	− 2	+ 1	− 2	+ 4	+ 4
	2	5.9	− 3	−10	− 1	− 4	+ 1	+ 1
		8.8	− 5	− 6	− 5	−11	− 2	− 3
		11.8	− 3	−10	− 4	−11	− 2	− 2
		D. Exposure of 8 hours						
Alsike clover	1	6.2	+ 4	0	+ 7	+ 4	− 7	0
		9.1	− 2	− 1	− 4	− 3	− 8	− 4
		12.2	− 4	− 8	+ 4	− 2	− 6	− 5
	2	6.2	− 1	−11	− 2	+ 1	− 5	− 3
		9.1	− 4	− 3	+ 3	+ 6	+ 1	− 7
		12.2	− 3	+ 3	0	0	+ 1	− 9
Ladino clover	1	6.1	0	− 1	− 1	− 1	+ 3	− 2
		9.3	+ 1	0	+ 3	− 2	+ 4	+ 1
		11.7	− 4	− 2	− 1	− 1	− 4	− 8
	2	6.1	+ 4	0	+ 5	+ 5	+ 2	0
		9.3	+ 2	+ 5	+ 1	+ 3	− 1	+ 1
		11.7	+ 2	− 3	+ 4	+ 1	− 6	− 3
Kenland red clover	1	5.9	+ 5	+ 3	+ 1	+ 3	− 3	− 2
		8.8	+ 1	+ 2	+ 3	− 1	+ 1	+ 2
		11.8	+ 2	+ 6	+ 3	+ 1	− 4	− 1
	2	5.9	+ 2	− 2	− 6	− 3	− 5	0
		8.8	− 1	− 3	− 5	− 4	+ 1	− 1
		11.8	− 2	− 5	− 3	− 3	+ 2	− 1

[a] Mean differences in the percentage of normal seedlings in germination tests with fumigated seeds from corresponding tests with unfumigated control samples; + indicates a greater, − less, and 0 the same percentage of normal seedlings in the fumigated as in the unfumigated controls.

[b] The exact dosages used for each exposure period at the temperatures indicated may be found in Table 1.

exposed to methyl bromide under all of the conditions listed in table 1, similarities between results obtained in some tests makes it unnecessary to present a summary of all of the data accumulated. Those data not presented are mentioned briefly, however, as equivalent tabulated information is given in this report.

Germinations of Ranger alfalfa seeds exposed to methyl bromide for 2, 8, and 24 hours were comparable and results in table 2, C for the latter exposure periods are representative of all three periods. Results from germination tests with seeds of alsike clover, ladino clover and Kenland red clover after fumigation with methyl bromide for 2 hours were similar to results summarized in table 2 from tests with seeds fumigated for 8 hours. Tolerance of Viking birdsfoot trefoil to methyl bromide fumigation indicated by the data in table 2 from tests with seeds exposed to methyl bromide for 72 hours at various temperatures and dosages was found in tests with seeds fumigated at shorter periods. This brief description of results obtained from germination tests, but not presented in table 2, indicates differences between species in susceptibility of small legume seeds to methyl bromide and the influence of exposure periods in contributing to injury during fumigation under the conditions of the study.

Injury was recognized by reduced percentages of germination in seeds of Ranger alfalfa, ladino clover, Kenland red clover, and alsike clover fumigated with methyl bromide under some conditions. Where reductions are found in table 2, probably variables of most importance in contributing to injury of seeds during fumigation were length of exposure to fumigant, dosages of fumigant, moisture content of seeds, and repeated fumigations.

Exposure of Ranger alfalfa, ladino clover, Kenland red clover, and Viking birdsfoot trefoil with graded moisture ranges approaching 6%, 9%, and 12% to one and two fumigations with 2 pounds of hydrocyanic acid per thousand cubic feet for 72 hours did not result in decreases in germination which could be attributed to hydrocyanic acid fumigation (table 3). Failure of hydrocyanic acid to cause injury under the fumigation conditions described indicated that seeds of these species are tolerant to the fumigant, and further tests were unnecessary. Tests with alsike clover seeds fumigated under the same conditions resulted in decreases in germination more severe than injury found after fumigation with methyl bromide. For this reason, alsike clover seeds were fumigated with hydrocyanic acid under all of the conditions given in table 1. After each final fumigation, seeds were aerated for 3 weeks, planted in fine vermiculite, and held at 68° F. for germination in facilities designed to prevent any interference between fumigant released from exposed seeds and control samples being germinated. Injury to germination indicated by results summarized in table 4 undoubtedly was caused by fumigation with hydrocyanic acid.

Table 3.—Variations[a] in the percentage germination of fumigated from unfumigated small legume seeds after one and two fumigations for 72 hours at 90° F. with 2.0 pounds of hydrocyanic acid per 1,000 cubic feet.

Kind of Seed	Moisture Content of Seeds (%)	No. of Fumigations 1	2
Ranger alfalfa	5.8	+3	−3
	8.9	+3	−1
	12.0	−1	+3
Ladino clover	6.1	+1	+1
	9.3	+3	−2
	11.7	+1	−2
Kenland red clover	5.9	−2	+3
	8.8	−3	+1
	11.8	−1	−1
Viking birdfoot trefoil	5.8	−8	+6
	9.1	+1	−1
	11.7	−4	−6

[a] Same as in table 2.

Differences in susceptibility to methyl bromide and hydrocyanic acid observed between species in germination tests with small legume seeds in this study should not be attributed to differences in seed quality, although quality or vitality of seeds indicated by germination of

Table 4.—Variations[a] in the percentage germination[b] of fumigated from unfumigated alsike clover seeds after one and two fumigations with hydrocyanic acid for 72 hours at three temperatures and varying dosages.

No. of Fumigations	Moisture Content of Seeds (%)	Fumigation Temperature and Dosages in Pounds per 1,000 Cubic Feet					
		90° F.		70° F.		50° F.	
		1.0	2.0	1.0	2.5	1.0	3.0
1	6.2	−17	−20	−20	−21	− 6	−10
	9.1	−21	−24	−24	−21	−12	−23
	12.2	−17	−29	−19	−17	−13	−12
2	6.2	−22	−22	−24	−24	−17	−22
	9.1	−31	−27	−25	−22	0	− 9
	12.2	−31	−25	−25	−27	−33	−32

[a] Same as in table 2.
[b] In vermiculite.

control samples must be considered as an important factor which may accentuate the susceptibility of seeds to fumigants under various conditions. As previously mentioned, differences between species in their response to fumigants were not in the same order as observed in results from germination tests with unfumigated seeds. Data presented in earlier reports by Lindgren *et al.* (1955) and Cobb (1958) supports the conclusion that susceptibility of small legume seeds to injury by fumigation with methyl bromide and hydrocyanic acid may vary between species, and the degrees of variation may range from complete tolerance to reductions in germination of economic importance.

Reductions in the percentages of germination of small legume seeds were caused by fumigation under some conditions, but complete destruction of the germinative capacity of seeds did not occur in any of the tests. This suggests that small legume seeds susceptible to methyl bromide and hydrocyanic acid may be fumigated to eradicate khapra beetle infestations without causing serious injury to seed germination by avoiding fumigation of seeds with high moisture contents and repeated fumigations. The use of minimum exposures and dosages of fumigant is indicated.

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Residues in Tissues and Eggs of Poultry Dusted with Co-Ral (Bayer 21/199)[1]

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Abstract

Thirty White Leghorn hens were dusted with P^{32}-labeled Co-Ral® (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothioate or Bayer 21/199) at 50 mg./kg. Twenty hens were dusted once, while 10 hens received two applications at weekly intervals. Some intact Co-Ral remained on the feathers and skin for 28 days after treatment. The liver, kidney, and bone contained more radioactivity than other internal tissues. By 3 days after treatment, acetonitrile-soluble residues in internal tissues were negligible. The eggs contained minute quantities of acetonitrile-soluble residues at 5 to 7 days after treatment of the hens, but these residues were not characterized as Co-Ral or its oxygen analog. Radioactive materials excreted in the feces consisted of residual Co-Ral, the oxygen analog, *O,O*-diethyl phosphoric and *O,O*-diethyl phosphorothioic acids. Phospholipids, ribose nucleic acid, desoxyribose nucleic acid and acid-soluble phosphorus compounds were isolated from the liver and feces.

The residues and metabolism of Co-Ral® (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothioate or Bayer 21/199) in mammals have been investigated by several research groups (Krueger *et al.* 1959, Robbins *et al.* 1959, O'Brien & Wolfe 1959, Kaplanis *et al.* 1959). Co-Ral is an excellent insecticide for endo- and ectoparasite control of cattle (McGregor *et al.* 1954, Smith & Richards 1954). The control of poultry insects with Co-Ral is quite feasible and has been demonstrated against certain species (Kraemer 1959).

The study reported here concerns the deposition of residues in tissues and eggs, and characterization of metabolites in the excreta of hens receiving one or two dust applications of P^{32}-Co-Ral.

Procedure.—*Materials.*—P^{32}-labeled Co-Ral (6.25 gm., 2 mc./gm. as of May 5, 1959) was synthesized by Farbenfabriken Bayer and provided for this study through the courtesy of the Chemagro Corporation. The P^{32}-labeled material was identical to an analytical stand-

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ard sample of nonradioactive Co-Ral as determined by infrared analysis, melting point, chromatographic behavior on Celite, and Rf values on paper chromatograms. Other materials used in this study were the oxygen analog of Co-Ral (Chemagro Corporation), *O,O*-diethyl phosphorothioic acid (American Cyanamid Company), and *O*-ethyl and *O,O*-diethyl phosphoric acids (Victor Chemical Company).

Application of Co-Ral Dust.—Thirty-three White Leghorn hens about 18 months old were housed singly in wire cages and were provided with laying mash and water free-choice. Egg production of the hens was 50% to 60% and the average weight of the hens was 2.0 kg.

Five grams of P^{32}-labeled Co-Ral was added to a one-half gallon vessel containing 495 gm. of dust base (supplied by the Chemagro Corporation) and thoroughly mixed on a tumbling machine for 4 hours until the ingredients were homogeneous as determined by radioassay. A 16-ounce polyethylene wash bottle was filled with the 1% Co-Ral dust, and each hen was dusted individually with 10 gm. of the material. This amount of dust was equivalent to 50 mg. of Co-Ral per kg. of body weight. Puffing the dust through the small opening of the wash bottle into the feathers was adequate to evenly distribute the dust to all portions of the body, except the head which was not dusted. The hens were held over a metal container during the dusting operation to collect the excess dust.

The hens were divided into three groups: three hens were used as untreated controls, 20 hens were dusted once, and 10 hens were dusted twice at weekly intervals.

Tissues and Eggs.—Three hens were sacrificed at 1 (2 hens), 3, 7, 10, 14, 21, and 28 days following the initial application of Co-Ral dust. The 10 hens receiving two dust applications were sacrificed in groups of 2 or 3 hens at 7, 14, 21 (2 hens), and 28 (2 hens) days after the second application. The three control hens were used to determine the percentage recovery of Co-Ral added to the tissues.

Immediately prior to sacrificing a hen, 5 ml. of blood was withdrawn from the heart region with a 10 cc. syringe containing a few drops of heparin. From 5 to 10 gm. of feathers and about 10 gm. of skin were removed from the back of the hens after sacrificing. The carcass was scalded to remove feathers and partially remove the dust from the skin to prevent contamination during dissecting. The liver, kidneys, gizzard, drumstick, breast, brain, samples of fat, and a portion of the leg bone were removed. Duplicate 1-gm. samples were radioassayed; some tissue samples were wet-ashed with HNO_3 for comparison of the total P^{32} residues. The remaining portion of the carcass of several hens was also wet-ashed for determination of total P^{32} residues.

Tissues containing greater than 0.02 p.p.m. were fractionated to determine the amount of acetonitrile-soluble residues. From 10 to 20 gm. of most tissues were used with duplicate analyses per tissue; the entire kidneys and brain were analyzed. Tissue preparation and extraction procedures were essentially the same as described by Anderson *et al.* (1959): acetone followed by benzene extraction of tissue macerates (Virtis Macrohomogenizer), partition of residue between water and *n*-hexane, and finally cleanup of the *n*-hexane-solubles with acetonitrile. Blood samples were extracted directly with *n*-hexane and then the *n*-hexane was partitioned with acetonitrile. Residues of Co-Ral or the oxygen analog were calculated from the radioactivity in the acetonitrile fraction, hydrolytic products from the water fraction, phospholipids from the *n*-hexane-solubles, and "unextractables" from the radioactivity remaining in the tissue residue.

Eggs were collected daily and analyzed separately. The total P^{32}-containing materials in the yolk, white (albumin), and shell of eggs were determined from the amount of radioactivity in duplicate 500-mg. samples of each egg fraction. Toxic residues in egg yolk or white were determined, using the extraction procedure described for Co-Ral from tissues. Three to four yolks or whites were combined for analyses.

Feces.—The droppings were collected daily on a piece of protecto paper placed underneath each cage. The feces from each hen were weighed, mixed, and two 500-mg. samples were radioassayed. The radioactivity was partitioned between 3 ml. water and 3 ml. chloroform to derive the percentage hydrolysis.

For the isolation and characterization of fecal metabolites, all samples collected at 1, 2, 3, and 4 days after treatment of hens with one dust application and at 1, 2, and 3 days after treatment with 2 dust applications were combined. Duplicate 300-gm. subsamples of the combined fecal samples were removed, placed in 600 ml. acetone, and allowed to stand for about 12 hours. The acetone was decanted and 600 ml. water was added to the solid materials and allowed to stand another 12 hours. The aqueous layer was removed by filtration and added to the residue of the acetone extracts. The water was extracted six times with an equal volume of chloroform. The chloroform extracts were dried, concentrated to an oily residue, and the residue was partitioned with an equal volume of *n*-hexane and acetonitrile.

The acetonitrile-solubles were co-chromatographed on a Celite column, using the solvent system of Vickery & Arthur (1960). Known materials were placed on the column with the radioactive acetonitrile-solubles. The percentage residual Co-Ral, the oxygen analog, and other metabolites was calculated from the co-chromatographic behavior of the radioactivity with the carrier materials. Results obtained from the Celite partition columns were supported by Rf values of Co-Ral and the oxygen analog from paper chromatograms using the solvent system described by Robbins *et al.* (1959). The radioactivity was located by counting 1-cm. strips of the chromatograms.

The radioactive materials remaining in the water after exhaustive chloroform extraction were co-chromatographed on an anion exchange column (Plapp & Casida 1958), with nonradioactive *O*-ethyl and *O,O*-diethyl phosphoric and *O,O*-diethyl phosphorothioic acids. The water-solubles from Co-Ral degradation were also subjected to ascending paper chromatography using untreated paper with 85:15 acetonitrile: water as the mobile solvent.

The P^{32} materials remaining in the fecal residue after extraction with acetone and water were further separated into acid-soluble phosphorus compounds, phospholipids,

ribose nucleic acid and desoxyribose nucleic acid (Schneider 1945). This procedure was also used on the "unextractable" materials from the liver of certain hens.

Other Methods.—Phosphorous was determined colorimetrically as reduced phosphomolybdate following perchloric acid oxidation (Allen 1940). Radioassays were made with an end-window Geiger-Müller tube, and samples were corrected for background, progressive decay, and dilution. Infrared analyses were made with a Beckman IR-5 with the compounds dissolved in carbon tetrachloride, as liquid films or as KB_r pellets.

RESULTS.—*Recovery of Co-Ral Added to Tissues and Eggs.*—The recovery of Co-Ral added to the tissues of the three control hens ranged from 79% from the breast to 100% from the feathers (table 1). In most instances the recovery of Co-Ral in the acetonitrile fraction was greater than 90%, and the balance of the radioactivity was about equally distributed between the *n*-hexane, water, and tissue residue. The extraction procedure was considered adequate for removing Co-Ral from tissues (table 1) and would account for the oxygen analog of Co-Ral (Krueger *et al.* 1959).

Table 1.—Recovery of Co-Ral added to poultry tissues and eggs.[a]

TISSUE	P.P.M. ADDED	P.P.M. RECOVERED[b]	PER CENT RECOVERY
Blood	0.160	0.133	83.1
Brain	.548	.533	97.3
Breast	.180	.143	79.4
Drumstick	.364	.298	81.9
Fat	.420	.387	92.4
Feathers	.331	.331	100.0
Gizzard	.352	.331	94.0
Kidney	.524	.497	94.9
Liver	.409	.369	90.2
Skin	.400	.351	87.8
Egg yolk	.502	.455	90.6
Egg white	.438	.245	56.0

[a] Averaged from tissues of three hens with duplicate determinations per tissue; eight determinations on egg yolk and white.
[b] Represents acetonitrile-solubles.

Good recovery of Co-Ral from egg yolk was obtained, but the recovery from the white was considerably less (table 1). Most of the radioactivity not recovered in the acetonitrile fraction from egg whites was present in the water fraction. The portion of radioactive material partitioning into water from *n*-hexane could not be removed from the water with chloroform.

Residues in Tissues.—There was an initial rapid decline of total P^{32} residues from the feathers and skin of hens dusted once or twice with Co-Ral (table 2). More than half of the applied Co-Ral was gone from the feathers by 3 days after treatment. Of the internal tissues, the liver and kidney contained the highest accumulation of total P^{32} materials; the breast and drumstick contained only negligible residues by 3 days after treatment with one dust application or 7 days after two dust applications; very little phosphorus-containing residues were stored in the fat. Radioactive materials in the bone were considerably higher than most other tissues, probably because of the degradation of Co-Ral to inorganic phosphate and deposition in the bone. Residues of P^{32} could not be detected in the blood and brain 1 week after treatment with one or two dust applications. Some of the Co-Ral may have been ingested from the feathers, but there was no appreciable accumulation of residues in the gizzard (table 2). There was little difference in the total p.p.m. in tissues as determined by counting portions of whole tissues or by wet-ashing and then counting. When the remainder of the carcass (excluding tissues shown in table 2) was digested with HNO_3, total P^{32} residues were less than 0.02 p.p.m. at 7, 10, and 14 days following the first application of Co-Ral dust.

Fractionation of the tissues to determine the p.p.m. acetonitrile-soluble residues yielded the results given in table 3. The acetonitrile-solubles were classified as toxic residues, since good recoveries of Co-Ral were obtained in the acetonitrile fraction of the fractionation procedure. The acetonitrile-soluble materials may not have contained Co-Ral or toxic metabolites, but may actually have been composed entirely of normally occurring acetonitrile-soluble phosphorus compounds. Co-Ral underwent very little change on the feathers and skin (comparison of total residues in table 2 with the acetonitrile-solubles in table 3). By 3 days after treatment, the gizzard, liver, and kidney were the only internal tissues having detectable toxic residues, and these residues were near the lower limit of detectability. Toxic residues in tissues following two dust applications of Co-Ral at 1 week intervals were about the same as those from one dust application (table 3).

Table 2.—Total p.p.m. Co-Ral equivalents in tissues of laying hens dusted with P-32 Co-Ral.[a]

TISSUE	P.P.M. CO-RAL EQUIVALENTS AT INDICATED DAYS AFTER TREATMENT										
	One Application							Two Applications			
	1[b]	3	7	10	14	21	28	7	14	21[b]	28[b]
Blood	0.064	0.033	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020	<0.020
Bone	.096	.106	.210	.182	.172	.155	.062	.332	.302	.216	.219
Brain	.054	.034	<.020	<.020	<.020	<.020	<.020	<.020	<.020	<.020	<.020
Breast	.030	<.020	<.020	<.020	<.020	<.020	<.020	.045	.032	.024	<.020
Drumstick	.044	.029	<.020	<.020	<.020	<.020	<.020	.032	.027	<.020	<.020
Fat	.086	<.020	<.020	<.020	<.020	<.020	<.020	.034	<.020	<.020	<.020
Feathers	631.79	259.19	94.22	88.28	25.17	34.00	3.10	94.52	71.90	42.41	8.84
Gizzard	.059	.035	<.020	<.020	<.020	<.020	<.020	.062	.030	<.020	<.020
Kidney	.802	.635	.566	.501	.333	.281	.152	.577	.424	.253	.127
Liver	.752	.842	.505	.374	.416	.462	.167	.478	.632	.407	.294
Skin	1.980	1.358	.586	.517	.231	.220	.156	1.034	.349	.238	.289

[a] One per cent Co-Ral dust applied May 15 and May 22, 1959, at approximately 50 mg./kg.
[b] Averaged from two hens, all other determinations averaged from three hens sacrificed at each time interval with duplicate analyses per tissue.

Table 3.—Acetonitrile-soluble radioactive materials in tissues of laying hens dusted with P-32 Co-Ral.[a]

Days After Treatment	P.p.m. Acetonitrile-Solubles in[b]—				
	Feathers	Gizzard	Kidney	Liver	Skin
One Application					
1	603.40	0.037	0.035	0.045	1.526
3	252.73	.023	.028	.024	.928
7	88.80	<.020	<.020	<.020	.359
10	88.28	<.020	<.020	<.020	.373
14	24.00	<.020	<.020	<.020	.091
21	25.89	<.020	<.020	<.020	.151
28	2.72	<.020	<.020	<.020	.090
Two Applications					
7	87.37	.029	.037	.029	.838
14	55.34	<.020	<.020	.032	.327
21	35.64	<.020	<.020	<.020	.198
28	6.26	<.020	<.020	<.020	.176

[a] One per cent Co-Ral dust applied May 15 and May 22, 1959, at approximately 50 mg./kg.
[b] Blood, brain, breast, drumstick, and fat contained less than 0.02 p.p.m. except the drumstick (0.025 p.p.m.) and fat (0.036 p.p.m.) on the first day after one dust application and the breast (0.025 p.p.m.) at 7 days following the second application.

Table 4.—Fractionation of the unextractable radioactive materials from feces and liver residue.[a]

Tissue or Excreta	Days After Treatment	Per Cent Present as[b]—				
		Acid Soluble Compounds	Phospholipids	RNA	DNA	Residue
Liver	3	35.8	25.4	36.8	1.6	0.4
Liver	7	23.9	28.5	43.1	4.5	0
Feces	3	46.6	45.2	7.4	0	.8
Feces	7	44.2	23.4	12.7	9.1	10.6

[a] One per cent Co-Ral dust applied to hens on May 15, 1959, at approximately 50 mg./kg.
[b] Fractionation procedure of Schneider (1945).

The acetonitrile-soluble residues (table 3) represents only a small portion of the total P^{32} residues (table 2). Most of the remaining P^{32} materials remained in the tissue residue. The separation of these unextractable P^{32} materials in the liver residue of certain hens into acid-soluble compounds, phospholipids, RNA (ribose nucleic acid) and DNA (desoxyribose nucleic acid) yielded mostly RNA, followed by acid-soluble phosphorus materials, and then phospholipids (table 4). Very little P^{32} materials remained in the liver residue and the DNA constituted only a minor fraction.

Residues in Eggs.—The yolk of eggs contained higher total P^{32} residues than the white or shell (table 5). The highest concentration of P^{32} materials appeared in the yolk of eggs at 6 to 9 days after treatment with Co-Ral, whereas the p.p.m. total P^{32} in the shell or white was almost nondetectable by 9 days after treatment.

Toxic residues (acetonitrile-solubles) were not present in the white of eggs from hens receiving one or two dust applications of Co-Ral; however, recovery of Co-Ral added to egg white was low (table 1). Toxic residues in the yolks was indeed minute (table 5), never exceeding 0.036 p.p.m. which was bordering the lower limits of sensitivity of the method. Most of the P^{32} materials in the yolk were unextractable from the yolk residue.

Excretion Rates and Hydrolysis in Feces.—About 11% and 27% of the applied Co-Ral was found in the feces by 28 days following the first and second application of dust, respectively (fig. 1). More than half of these radioactive materials appeared in the feces by the first 24 hours after treatment. The cumulative per cent excreted (fig. 1) is not a true approximation of the amount of the applied Co-Ral which was absorbed and eliminated in the excreta. Some Co-Ral dropped from the feathers onto the feces and some of the dust may have been ingested.

Co-Ral was recovered quantitatively in the chloroform layer when added to control feces and partitioned between chloroform and water. Percentage hydrolytic products based on partition coefficients ranged from 10% the first day following treatment to 74% on the fifth day. Results on the percentage hydrolysis was erratic due to poor sampling technique and contamination of the droppings with the dust that dropped from the feathers.

Table 5.—P.p.m. Co-Ral equivalents and acetonitrile-solubles in eggs from hens dusted with Co-Ral.[a]

Days After Treatment	Total P.p.m. Co-Ral Equivalents						P.p.m. Acetonitrile-Solubles	
	One Dust			Two Dusts[b]			One Dust	Two Dusts
	White	Yolk	Shell	White	Yolk	Shell	Yolk	Yolk
1	<0.020	<0.020	0.045	0.025	0.392	0.118	<0.020	0.030
3	<.020	.070	.028	.056	.471	.312	<.020	.034
4	.040	.073	.054	.055	.476	.151	<.020	.027
5	.052	.198	.214	<.020	.414	.048	.036	.026
6	.048	.232	.059	.029	.416	.073	.032	<.020
7	.032	.364	.082	.037	.492	.068	.027	<.020
8	.069	.335	.036	.026	.398	.071	<.020	<.020
10	<.020	.257	.021	<.020	.310	.021	<.020	<.020
12	<.020	.163	<.020	<.020	.251	.029	<.020	—

[a] One per cent Co-Ral dust applied on May 15 and May 22, 1959, at approximately 50 mg./kg.
[b] Days after the second application of dust.

Table 6.—Fractionation of the P-32 Co-Ral and metabolites recovered from the feces of laying hens receiving one or two applications of Co-Ral dust.[a]

Days After Treatment	No. of Applications	Per Cent Present as—					
		Chloroform-Celite[b]			Water-Anion[b]		
		Co-Ral	O-Analog	MeOH	$C_2H_5OP(O)(OH)_2$ + $(C_2H_5O)_2P(O)OH$	$(C_2H_5O)_2P(S)OH$	Feces Residue
1	1	91.53	0.32	0.81	1.35	1.18	4.81
2	1	58.59	0.52	1.78	9.22	6.96	22.93
3	1	65.12	0.63	1.12	7.48	3.91	21.74
4	1	71.66	0.75	0.0	7.95	4.20	15.44
1	2	87.98	0.23	0.39	4.25	3.97	3.18
2	2	95.01	0.25	0.52	1.15	0.69	2.38

[a] One per cent Co-Ral dust applied on May 15 and May 22, 1959, at approximately 50 mg./kg.
[b] Identifications based on co-chromatographic behavior on Celite or anion exchange columns.

This method of determining percentage hydrolytic products did not take into account the unextractable P^{32} materials from the feces.

Fractionation of Fecal Metabolites.—Duplicate 300-gm. subsamples of feces collected at various intervals after treatment were extracted with acetone, then with water, and the combined aqueous phase partitioned with chloroform. More than 60% of the radioactive materials partitioned from water into chloroform and about an equal amount remained in the water and feces residue.

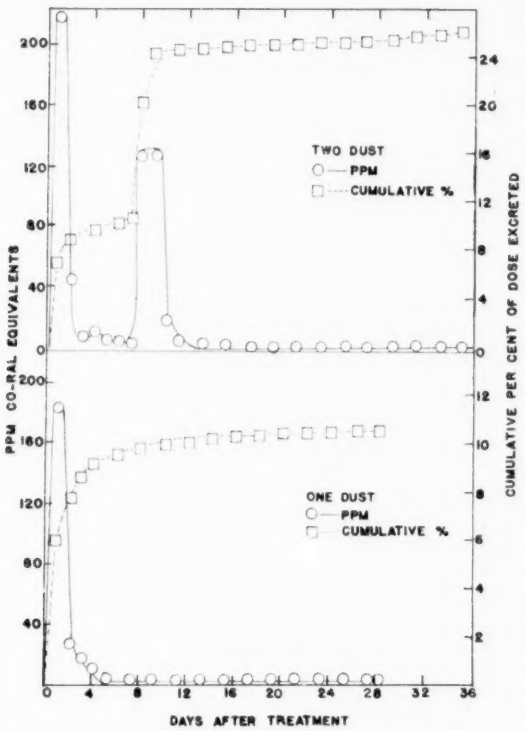

FIG. 1.—P.p.m. Co-Ral equivalents and cumulative per cent of total administered dose excreted in feces of hens dusted with P^{32} Co-Ral at 50 mg./kg.

The chloroform-solubles were further partitioned between *n*-hexane and acetonitrile and the acetonitrile fraction co-chromatographed on Celite with nonradioactive Co-Ral and the oxygen analog, while the water-solubles were subjected to anion exchange chromatography (table 6). Most of the chloroform-solubles chromatographed as Co-Ral; the oxygen analog represented less than 1% of the total radioactive materials in the feces. Results from the Celite columns (table 6) were substantiated by Rf values of Co-Ral (0.573) and the oxygen analog (0.187) on paper. Slightly more mono- and diethyl phosphoric acids resulted from Co-Ral degradation than the phosphorothioic acid (table 6). Separation of the water-soluble metabolites of the feces by paper chromatography was somewhat less efficient than the anion exchange columns because of extraneous materials in the fecal extracts.

Fractionation of the unextractable P^{32} materials from the fecal residue (table 6) of certain hens is given in table 4. Most of the radioactivity was in the acid-soluble and phospholipid fractions.

DISCUSSION.—Residue data based on total radioactive materials in tissues are often misleading, since no consideration is given to the nature of the residues. Isolation of toxic residues is usually based on selective solvent extraction procedures, which give good recoveries of known amounts of the insecticide when added to tissues. Even though about 90% of the Co-Ral added to poultry tissues was recovered in the acetonitrile fraction (table 1), all radioactive materials in the acetonitrile fraction cannot be classified as Co-Ral or the oxygen analog. Other acetonitrile-soluble metabolites, which did not have the chromatographic behavior of Co-Ral or the oxygen analog, were isolated from poultry feces (table 6, MeOH fraction) and from rat excrement (Vickery & Arthur 1960). A comparatively innocuous metabolite of Co-Ral degradation, des-ethyl Co-Ral, has been isolated from mammals (Krueger *et al.* 1959), and was suggested as a possible constituent of the MeOH fraction from Celite columns of rat excrement (Vickery & Arthur 1960). Thus, the acetonitrile-solubles classified as toxic residues in tissues (table 3) and eggs (table 5) could represent other radioactive materials other than Co-Ral or the oxygen analog. Lack of correlation in the disappearance of acetonitrile-soluble residues from tissues (table 3) and

eggs (table 5) is further evidence that the acetonitrile fraction contained some other metabolite. Similar results have been noted with malathion (March *et al.* 1956).

The low recovery of Co-Ral from egg white (table 1) may be the result of protein binding (Krueger *et al.* 1959), but protein binding was not an apparent factor involved in similar *in vitro* studies with egg white (Anderson *et al.* 1959). The nature of the unextractable P^{32} materials from tissue residues after the appropriate solvent extraction has received very little attention. The normal phosphorus metabolic pool is tremendous and the complete degradation of organophosphates to phosphoric acid, which is incorporated into normal phosphorus-containing metabolites, partially explains the large amount of unextractable P^{32} materials from tissues (table 4).

No symptoms of poisoning were observed in hens receiving one or two dust applications of Co-Ral. Egg production and feed consumption was the same after treatment as before. Apparently Co-Ral as a 1% dust was less toxic to the adult hen than a 0.25% suspension of Co-Ral was to baby chicks (Kraemer 1959).

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The Role of Attractants in the Recent Mediterranean Fruit Fly Eradication Program in Florida[1]

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ABSTRACT

The invasion of Florida by the Mediterranean fruit fly (*Ceratitis capitata* (Wied.)) in 1956 was similar to that in 1929 in that the insect was initially discovered in April in grapefruit and eradication was completed in 18 to 19 months. The eradication programs were quite different however, the manpower required in 1956–57 being more than 85% less, because of the availability of highly effecture lures, plastic traps and bait sprays.

Eradication was achieved with a minimum of removal and destruction of ripening fruit and little adverse effect on Florida's agricultural economy. The sprays also greatly suppressed house flies (*Musca domestica* L.) and mosquitoes. Some species of tropical fish in very shallow water were susceptible to the small amounts of malathion in the bait spray. Other authentic cases of injury to persons, livestock, birds or pets were not discovered. Lacquer-type automobile finishes were injured when spray droplets were not washed off soon after deposition.

When the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), (often referred to as medfly) reappeared in Florida in 1956 after an absence of 26 years following its eradication in 1929–30, the on-tree annual value of the citrus crop had increased to about 250 million dollars and that of all other commercial hosts in Florida was many million dollars more. It was estimated that if the fly were not eradicated the combined annual cost of control efforts, fumigation of exported fruit, and maintenance of quarantine and road blocks would approximate 20 million dollars, exclusive of crop losses and adverse effects on the tourist industry and consumer acceptance of the State's citrus products.

Quick action by State and Federal agencies and industry, along with good public support, made it possible to set up effective containment and eradication programs in a minimum of time (Agricultural Research Service,

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Plant Pest Control and Entomology Research Branches 1956), and the second eradication effort has been a complete success.

This report presents data illustrating the effectiveness of the methods, primarily those concerned with the use of attractants for detection and eradication of the infestation, apart from those used to prevent or retard spread.

Two Eradication Campaigns Compared.—The differences and similarities in the two eradication campaigns have been discussed by Ayers (1957) and Rohwer (1958). In both cases the insect was found in April, in grapefruit, and eradication was considered complete after about 18 or 19 months.

The problems were different in the two invasions. In 1929 the infestation occurred chiefly in rural areas, including extensive citrus acreages. Orlando was the largest city involved. In 1956 the fly was first discovered in Miami, and much of the campaign had to be carried on in such thickly populated areas as Miami, St. Petersburg, and Tampa.

In 1929–30 some trapping of flies was done with about 12,000 glass traps baited with kerosene or a fermenting solution, but major dependence for information on fly distribution was placed on cutting and examining the fruit. In 1956–57 most of the infestations were located and progress towards eradication was judged by fly captures in traps. More than 50,000 plastic traps baited with several different attractants were used. Some very intensive fruit cutting was practiced to some extent, chiefly to locate sources of infestation near adult finds. This was done mostly during the later stages of the eradication program. However, at very low levels fruit cutting to detect infestation, even when restricted to favored hosts, was practically useless.

In 1929–30 eradication was chiefly by destruction of fruits and vegetables, with some removal of trees and some spraying from the ground with lead arsenate plus sugar, syrup, or molasses. In 1956–57 bait sprays of malathion plus protein hydrolysate were applied by airplane. In about one-twentieth of the infested area a soil treatment, mostly with dieldrin, was also used. General removal and destruction of infested fruit would have been very expensive, if not impractical, because of the greater prevalence of such fruits as Surinam cherry, calamondin, mango, and guava and the great increase in labor costs. It was done only on a few isolated, heavily infested, favored trees after bait sprays had been applied. Observations in Hawaii have strongly indicated that the absence of ripening fruit stimulates dispersal of flies from their emergence sites. This does not apply to the destruction of dropped fruit, which is largely ignored by the flies.

New fumigation treatments involving use of methyl bromide and ethylene dibromide (Balock 1951, Balock & Lindgren 1951, Richardson & Balock 1959) permitted almost normal harvesting and movement of the most susceptible crops with little loss to agriculture. The availability of ethylene dibromide treatment schedules for citrus developed by Miyanooto McPhail, W. E. Stone, and associates in Mexico was an important factor.

About 6,000 employees were engaged in the 1929–30 campaign; only about 800 were required in 1956–57. Eradication in 1929–30 cost more than 7 million dollars, plus many millions more in crops destroyed or inability to ship crops to market. In 1956–57 the same objective was reached at a cost of less than 11 million dollars (not adjusted for differences in buying power). Losses by crop destruction or inability to ship fruit or vegetables to market were insignificant.

The eradication of the medfly in 1956–57 with a much smaller working force than in 1929–30 and without destruction of fruit and other practices costly to the industry and irritating to the general public was possible because of the research carried on in Hawaii (Carter 1950, 1952, Christenson *et al.* 1956, Christenson 1958) and elsewhere. Among the most important results of research were the development (1) of highly effective lures for use in bait traps as indicators of the presence of the flies in a given location and as an index to the progress toward eradication, and (2) of attractive materials to be combined with insecticides in bait sprays.

LURES AND DETECTION METHODS.—Less than 2 months before the 1956 finding of the medfly in Florida, members of the laboratory in Hawaii had discovered that angelica seed oil is an outstanding lure for this fly (Steiner *et al.* 1957). It attracts only male flies. This material is produced in limited quantities, chiefly for use in perfumes and flavoring extracts. For large-scale trapping a plastic trap was designed (Steiner 1957a), and more than 50,000 of them were ultimately put into service.

At first the plastic traps were baited with angelica seed oil contining 3% of DDVP, applied weekly to cotton dental-roll wicks at the rate of 0.5 ml. per wick. The DDVP was added to the oil to give a quick kill of flies entering the trap and to reduce the number that might leave the trap before dying. A 5% chlordane dust (later changed to a 1:1 mix of 25% lindane and 40% chlordane wettable powders) was applied biweekly at the rate of one-fourth teaspoon per trap to prevent ant and spider depredation and assist in fly kill. Variability in the performance of different lots of angelica seed oil made it necessary to test them in the field in Hawaii before purchase. Use of aluminum foil wraps on the wicks to retard volatilization, as suggested by Simanton (1958), improved the performance of some of the fresh oils purchased in 1957.

By the end of 1956, after purchase of approximately 800 pounds of angelica seed oil had temporarily exhausted the world supply, the isopropyl and *sec*-butyl (siglure) esters of cyclohexene carboxylic acid (Gertler *et al.* 1958) were substituted, except in the most critical areas. They were generally used at the rate of 3 ml. per wick per week, supplemented with 0.25% to 0.5% of DDVP. At temperatures above 70° F. applications of siglure with a high trans-isomer content, which became available in the second half of 1957, at six times the rate of angelica seed oil equaled or exceeded the latter in effectiveness (Steiner *et al.* 1958b). As falling temperatures approached 60° siglure lost effectiveness more rapidly than angelica seed oil.

During the last few months of the campaign, when trap servicing was on 2- and 3-week schedules, lure application rates were adjusted to avoid complete volatilization before time for retreatment. The traps were placed at densities of 10 to 40 per square mile, preferably in medfly hosts. The number was always increased after

either a larval or adult find. Early in the trapping program, before adequate numbers of traps were available, it was necessary to use less than 10 per square mile in most of the susceptible area.

Recent studies in Hawaii have shown that even at a density of 40 traps per square mile not more than 1 of 25 emerging flies is likely to be caught in traps baited with either angelica seed oil or siglure. Recurrent infestations in Florida, after as much as 2 months had elapsed between fly catches, made it evident that even with the best available lures the medfly population could survive at population densities below levels that were immediately detectable. In some situations, possibly where conditions were not favorable for maximum reproduction, three generations appeared to be necessary for the flies to regain numbers that would have a reasonable chance of detection by the trap distribution employed.

Florida areas difficult of access, such as the Everglades, Ten Thousand Islands, river bottoms, and forests, were surveyed for hosts by helicopter, light plane, swamp buggy or boat. Traps were placed in or near all known host areas. Several trap lines in the Ten Thousand Islands which were run by helicopter or boat, revealed a number of infestations.

After the discovery of infestations, and their apparent eradication, intensified trapping was continued for a year or more. The date of the last fly catch (or larval find) determined the date of cessation of spraying and removal of quarantine restrictions.

BAIT-SPRAY USAGE.—Under Hawaiian conditions bait sprays containing a protein hydrolysate and an organic-phosphorus insecticide (Steiner 1952, 1955, 1957b, Steiner *et al.* 1958a) have given nearly complete or complete kill of all fruit flies within a few hours. Three species were involved, including the medfly. Less than half as much toxicant was needed when the bait spray was used instead of conventional sprays, with consequent less hazard from residues and injury to beneficial insects. The bait sprays were fed on by flies in all stages of development. They have been found most effective when applied uniformly, but with no more water than necessary to effect proper dispersal (Steiner *et al.* 1958a). In May 1956 personnel of the Agricultural Research Service Aircraft and Special Equipment Center found that the recommended 1 pound of protein hydrolysate solids plus 2 pounds of 25% malathion wettable powder per acre could be dispersed satisfactorily by aircraft in as little as 1 gallon of water. At first sprays were applied on a 10-day schedule, but after the acreage under treatment declined a 7-day schedule was adopted better to contend with rainy conditions that affected their residual effectiveness. The shortened interval also provided less opportunity for escape of flies from the few remaining infestations. In the 7-day schedule the application rate was reduced to 0.6 pound of protein hydrolysate solids plus 1.2 pounds of 25% malathion wettable powder per acre.

Bait-spray applications were made either from single-engine planes flying 75- to 100-foot swaths at 50- to 100-foot elevations or from 2- or 4-engine planes flying 225- to 500-foot swaths at 150 feet. The largest plane, a converted B-17 bomber, carried up to 2,400 gallons and covered 2,400 acres in about 15 minutes. Most of the spraying was done early in the morning before winds or high temperatures caused too much drift, dusting of deposit, or hazard in low-level flying. Wet deposit droplets from $\frac{1}{32}$ to $\frac{1}{8}$ inch in diameter were desired. In contrast to the usual aerial-spray practice, the planes flew across the wind beginning at the windward side and turned toward rather than away from the spray drift. The purpose of this procedure was to utilize the attraction of the spray to deplete fly populations ahead of the advancing front so that later there would be few flies to move upwind into areas already sprayed if the deposits had in the meantime been depleted by rain or other factors. Areas were resprayed only when more than 1 inch of rain occurred within 2 hours after an application.

PRELIMINARY TESTS OF BAIT SPRAYS IN MIAMI SHORES.—At the beginning of the campaign it was essential to determine the effectiveness of the bait sprays at the high temperatures that occur in Florida, for in Hawaii temperatures rarely exceed 85° F. It was also necessary to find out the effectiveness of a highly concentrated bait spray applied by air at the rate of 1 gallon per acre in areas where overhanging eaves and dense shade trees would shield many preferred hosts.

A 300-acre block in Miami Shores, near the site of the original medfly find, was divided into three plots, each 1,800 feet wide and 2,400 feet long. Results were evaluated with glass invaginated traps baited with a liquid lure containing 5% of protein hydrolysate plus 5% of ammonium chloride, or with angelica seed oil on a cotton wick. The protein hydrolysate lure was used to determine whether female flies were reaching sexual maturity between spray dates. The traps were placed in or near hosts in the backyards of homes along the middle of each plot more than 450 feet from the borders. Additional traps were placed one-half mile to the east, west, and south of sprayed areas to serve as controls. The traps were operated for 4 days before the single application of spray was made, at the rate of 100 gallons per plot. The spray treatments included 2 pounds of 25% malathion wettable powder, alone, with an acid hydrolysate of corn protein (SIB-2[6]), and with an enzymatic yeast hydrolysate. The sprays were applied on May 23 at a temperature of 88° F. in a 6 m.p.h. wind.

The results of these tests are shown in table 1. Those with the malathion-SIB-2 bait spray were especially significant, since the pilot inadvertently missed the two middle swaths in which all the traps in this plot were located. Despite continued heavy emergence from these missed swaths, this treatment was highly effective throughout the period. A similar condition existed in the malathion-yeast hydrolysate plot, part of which was also missed. Malathion alone required 4 to 5 days to attain its maximum effect owing to the time lag before the flies contacted the poison. The flies responded to the two protein hydrolysate sprays almost immediately. Even though rains on the second and third days removed most visible deposits and shade trees, overhanging eaves, and the missed 200-foot strip interfered with deposition on many host plants, the bait-spray treatments performed

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Table 1.—Reductions in medfly catches in preliminary bait-spray tests, Miami Shores, Florida.

Treatment	Number of Flies per Trap-Day Before Spray	Per Cent Change in Number of Flies Caught After Days Shown				
		1–3	4–5	6–9	10–16	2–11
Control:						
West	15.4	−48	−53	−85	−83	−71
East	3.5	+26	−14	−14	+14	0
South (100–200 yards)	7.1	−75	−61	−13	−32	−38
Malathion:						
Alone	13.2	−85	−89	−81	−75	−82
Plus enzymatic yeast hydrolysate	9.0	−99.1	−97.7	−94	−76	−92
Plus SIB-2	19.3	−99.8	−99.6	−98.6	−94	−98

Table 2.—Spray operations and medfly populations in greater Miami spray area, 1956.

Spray No.	Completion Date	Acres Sprayed (in thousands)	Number of Traps	Number of Flies per 1,000 Trap-Days	Remarks
Before spraying	—	—	56–477	3,000	May 17 to June 25
1	June 25	197	477	116	1 lb. enzymatic yeast hydrolysate/acre
2	July 7	210	600	42	1¼ to 2 lb. SIB-2, resprayed 35,000 acres because of rains
3	July 17	210	730	70	1¼ lb. SIB-2 or SIB-7/acre, supply short
4	July 27	210	765	2.2	1¼ lb. SIB-7/acre, supply short, rainy weather
5	Aug. 6	210	1,115	9.0	1¾ lb. SIB-7/acre, hurricane Betsy
6	Aug. 15	191	1,310	2.7	19,000 acres Miami Beach discontinued
7	Aug. 24	47	1,800	1.3	
8	Sept. 1	44	2,200	0.7	2 lb. SIB-7/acre, 65,000 acres, strip-sprayed
9	Sept. 15	41	2,400	.6	
10	Sept. 22	29	2,800	.25	Several noncontiguous areas remain
11	Oct. 2	31	2,900	.20	Single-engine planes on small areas
12	Oct. 9	21	3,000	.20	
13	Oct. 18	22	3,000	.25	Interrupted by high winds
14	Oct. 27	18	3,000	.06	Final spray for multi-engine planes
15	Nov. 1	9	3,000	0	Scattered small areas
16	Nov. 10	8	3,000	.03	

satisfactorily. Population depression in the west check was greatest after 6 to 9 days. This may have been caused in part by reduced downwind movement from the treated area. The prevailing winds were east-northeast, and catches in the traps 100 to 200 yards to the south quickly dropped to a low point.

ERADICATION IN GREATER MIAMI AREA.—The infestation in Miami, which was widespread and well established for several weeks before plans, supplies, contracts, and personnel could be assembled, provided the only opportunity for studies of eradication progress in relation to pretreatment populations (table 2). The first spray, on 197,000 acres from West Palm Beach to South Miami, was started on June 16 and completed on June 25. Only 477 traps were available prior to June 25, but they were well distributed in 12 lines throughout the greater Miami area. Additional traps were subsequently interspersed among the original 477. The prespray catch averaged 3,000 flies per 1,000 trap-days. Ninety-four per cent of the traps operated prior to July 31 caught flies. Catches in single residential lots ranged up to 1,500 flies.

Enzymatic yeast hydrolysate was unavailable in sufficient quantities for more than the first spray. The performance of SIB-2 in Miami Shores and its lower cost led to use of this bait in the second and third sprays, but the rapidly expanding acreage made it evident that the supply of this material also would be inadequate. Dosages were therefore reduced temporarily. Research in Hawaii had shown that corn steep liquor was very attractive, and after blends of SIB-2 and steep liquor were assayed in Honolulu, a 60:40 mixture, designated SIB-7, was selected. Application rates of SIB-7 also had to be held below the desired rate until adequate supplies became available in September, although deliveries of 10,000 gallons per day were maintained for several weeks.

Treatment of 19,000 acres of Miami Beach was discontinued after only five sprays. However, a series of recurrences on islands in Biscayne Bay and in part of Miami Beach indicated that the termination date for part of the area was premature.

At the start of the program a period of 4 weeks, or three sprays at 10-day intervals, was considered ample to span a medfly generation under south Florida conditions. However, soon after the first spray was completed it was discovered that larvae were being trapped in overripe sour orange and grapefruit (known as "shiners" in Florida) and that they could pupate and complete their development later. Further studies showed that larval life might be extended to as much as 30 days in these "shiners," and the life cycle to 50 days even under opti-

mum temperature conditions. It was also found that there was some retention of mature larvae in overripe mangoes and tangerines, but for shorter periods than in grapefruit and sour oranges. The spray program was modified in line with these findings.

Strip spraying of 41,000 to 47,000 of 65,000 acres was tried in three applications, but the results were not so good as when there was solid coverage. In this operation approximately 200-foot strips were left unsprayed between each two 500-foot swaths.

Bait-spray applications after the ninth were generally in areas where infestations had recurred or where all the numbered sprays had not been applied. During the course of the campaign fly populations declined to progressively lower levels after each generation. The overlapping of broods masked this trend to some degree, but the decline after each successive 24- to 39-day period was very evident (table 3).

STATE-WIDE ERADICATION.—The story of the eradication of the medfly over the entire State paralleled that in the greater Miami area. The overall eradication program has been or will be reported in detail by others, but its progress is evident from the data in table 4. The objective was reached in spite of occasional setbacks.

Table 3.—Medfly population decline by approximate generations in greater Miami spray area.

Period	Days	Spray Nos.	Approximate Generation	Mean Catch in 1,000 Trap-Days	Per Cent Reduction	
					Since Preceding Period	Cumulative
May 17–June 25	0–39	Prespray	Mixed	3,000	—	—
June 25–July 26	31	1–3	1	76	97	97
July 27–Aug. 23	27	4–6	1 and 2	4.6	94	99.099
Aug. 24–Sept. 21	28	7–9	2 and 3	0.9	80	99.970
Sept. 22–Oct. 26	34	10–13	2, 3, and 4	.22	76	99.993
Oct. 27–Nov. 20	24	14–16	3, 4, and 5	.03	86	99.999

Table 4.—Status of bait-spray and trap operations for eradication of the medfly in Florida.

Date or Period Ending	Counties Being Sprayed	Acres Sprayed by Air		No. of Traps in Use	Flies per 1,000 Trap-Days
		Currently	Cumulative Coverage		
1956					
June 30	19	328,305	495,541	4,000	122.500
July 31	24	602,381	1,996,000	17,000	7.490
Aug. 31	23	239,646	3,321,091	18,100	3.780
Sept. 30	19	215,506	4,022,141	34,157	0.882
Oct. 31	16	168,485	4,921,715	39,503	0.475
Nov. 30[a]	14	106,820	5,510,613	45,060	0.161
Dec. 31[b]	14	38,055	5,787,193	45,801	0.027
1957					
Feb. 28	11	24,580	6,168,696	45,626	0.037
Apr. 30	5	11,530	6,324,529	47,810	0.051
June 30[c]	6	31,100	6,572,925	48,760	0.033
Aug. 31	2	3,500	6,723,052	36,978	0.002
Oct. 31	1	1,400	6,747,592	27,757	0.001
Dec. 31	1	4,600	6,787,653	23,722	0.004
1958					
Feb. 28	0	0	6,805,000	25,197	0

[a] Hernando (last county found infested) added.
[b] Insecticide applications completed in all eastern and southern counties.
[c] Final eradication in all counties except Hillsboro, Lake, Manatee, Orange, Pasco, Pinellas, and Polk.

The general plan was to apply aerial sprays to all known infested areas and strips one-half mile outside these areas. However, expanses of water or open pasture that occurred near infested areas were not considered as part of the half-mile strip, since the flies moved rapidly across these treeless areas and it was necessary to include a reasonable area beyond them. Recurrences of infestation were found in less than 10% of the acreage after spraying ceased. Most of these recurrences probably resulted from inadequate trapping, weakness in coverage because of pilot error, applications delayed by rain or other factors, plane detours around obstructions such as high radio towers and oil- or gas-storage tanks, variable winds in some areas causing swaths to overlap or be blown widely apart, or premature termination of spraying. Standard practice was to continue spraying for a period long enough to span a complete generation after the last fly or larval find.

Several serious recurrences that spread to new acreage before they could be located developed in April and May 1957, apparently having built up during winter months when cool weather decreases the efficiency of the traps and extends the life cycle of the fly. One of these in Polk County, where there had been improper operation of traps, had reached a biologically explosive level and caused new infestation ½ to 2 miles away which added more than 10,000 acres to the treated area by the time all were discovered. Incidentally, this occurrence demonstrated again the fly's ability to thrive under the winter temperatures that prevail in central Florida, corroborating the findings of Messenger & Flitters (1954) in bioclimatic cabinet studies in Hawaii.

The last medflies known to exist in Florida were taken on Snead's Island at the mouth of the Manatee River on November 26, 1957. This infestation may have resulted from infested guavas or wild citrus floating downstream from the last previous find in September 5 miles upstream. Spraying was continued on Snead's Island until February 25, 1958, 13 weeks after the last fly was found. Intensive trapping was continued through most of 1958, and traps are still being maintained at strategic points. The results have all been negative.

More than 6 million pounds of protein hydrolysate solids and 12 million pounds of 25% malathion wettable powder were used in the eradication program.

SIDE EFFECTS OF BAIT-SPRAY APPLICATIONS.—There was considerable damage to lacquer automobile finishes as a result of the softening of the paint film under bait-spray spots (particularly on new cars) when deposits were not washed off promptly. Enamel finishes were unaffected. Investigations by chemists and paint research establishments indicated that malathion may have been responsible. A safe, effective toxicant is urgently needed before extensive urban areas are again sprayed from aircraft.

Some losses of fish in uncovered ponds were reported by tropical fish breeders. However, a series of tests indicated that most species can tolerate at least six to eight times the usual dosage of the bait spray when they are in 9 or more inches of water. Mortality of very small fish in water less than 3 inches deep was observed at some locations where applications were heavier than usual be-

cause of overlapping of spray swaths or low flying on windward shore lines to insure coverage of sea grapes and other vegetation along the water's edge. It was probably natural for the public to blame the aerial sprays for all mortality of birds, animals, and bees, as well as plant defoliation, failure to flower, and human illnesses. Many claims were investigated by the writers or other professional people in the subject fields involved. Other than those indicated above, no authentic cases of injury to animals, birds, beneficial insects, or the public either from the bait sprays or the use of DDVP in the traps were ever found.

INCIDENTAL OBSERVATIONS.—Unusually high trap catches of various insects have often been associated with approaching storms. Of the 204 flies caught in Miami during the 30-day period prior to August 15, 77 were taken on August 12–13, just before the predicted arrival on August 14 of hurricane Betsy. In Broward County 29 of 65 flies caught in the same period were also taken on August 13–14. The possibility that elements associated with but preceding this storm front may have been responsible for increased catches, through stimulation of greater flight activity or increasing distance of attraction or fly sensitivity to the lure is strongly indicated. These sudden increases in trap catches (which also occurred on the same dates elsewhere ahead of the advancing storm) provided a sharp warning that more flies were still present in the original infested area than had been indicated by earlier catches.

The sprays substantially reduced house fly and mosquito populations in the treated areas. Circumstantial evidence has also suggested that good control of the papaya fruit fly (*Toxotrypana curricauda* Gerst.) was obtained during periods while sprays were applied regularly.

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Control of the Tadpole Shrimp, *Triops longicaudatus*, in California Rice Fields[1]

A. A. Grigarick,[2] W. H. Lange,[2] and D. C. Finfrock[3]

Abstract

The tadpole shrimp is a common pest in most of the northern rice-growing counties of the Central Valley of California. It causes damage of two types: a chewing of roots and leaves, and a dislodging of rice seedlings by the chewing and by burrowing on the bottom. Field and laboratory tests conducted in 1954, 1957, 1958, and 1959 confirm that 2 pounds of DDT gives control when applied as granules with the seed, or application as granules or sprays after seeding. Copper sulfate "resistance" was associated with the rate of solubility of the bluestone crystals, for solutions were effective but crystals were not. Of the newer materials tried for shrimp control, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), malathion, and Sevin® (1-naphthyl methylcarbamate) were all effective at 2 pounds actual toxicant per acre as postflooding sprays.

Branchiopod crustaceans, of the order Notostraca, are common inhabitants of temporary pools of fresh or brackish water. California rice culture creates such a freshwater habitat. Rosenberg (1947) reported *Triops longicaudatus* (Le Conte) (under the names *Apus oryzaphagus* and *Apus biggsi*) to be damaging rice seedlings in Butte and Sutter Counties. Such damage has since been found in most of the northern rice-growing counties of the Central Valley of this state. Longhurst (1955), in a taxonomic review of this order, recorded the range of *T. longicaudatus* as Western North America south of 50° N., through Central and South America, West Indies, Galapagos Islands, Hawaii, Japan, and New Caledonia. It is also a pest of rice in Japan. Longhurst listed *T. cancriformis* (Bosc.) as a rice field pest in Northern Italy, Spain, and France; and Walton & Kemp (1911) reported the same species damaging rice in India.

T. longicaudatus is commonly called the "tadpole shrimp" by California rice growers because of its similarity to the true tadpole in color, size, and swimming activity (fig. 1). It is olive-gray, the anterior portion of the body is covered with a shield-like carapace, and the nearly cylindrical abdomen extends from beneath this carapace and terminates in two cercopods. Overall length may reach 2 to 2½ inches. The crustacean has two large compound eyes and a median ocellus. The first and second pairs of appendages are respectively the antennules and antennae, and the third pair forms two strong toothed structures called the mandibles, which may be compared to those of a chewing insect. Numerous appendages bearing leaf-like gills attach to the abdomen.

In late April and May the shrimp are easily observed within 8 to 12 days after infested rice fields are flooded. Rosenberg (1947) reported on the following phases of shrimp biology. Dried eggs from the previous year hatched 72 hours after water submergence. The metanauplii and succeeding stages passed through a series of moults until they became adults. Reddish-orange egg-sacks appeared along the sides of the abdomen near the carapace in about 8 to 10 days. The number of generations was not determined but was believed to be more than one because eggs taken from the egg-sacks and kept in water hatched in a minimum of 5 days. Longhurst (1955) reported that species of this genus occur only in waters that dry out regularly. He was able to induce hatching of nondried eggs in 12 to 14 days by reducing the osmotic pressure of the water, a condition not likely to occur at the bottom of a pool, where the eggs are laid. He reported that reproduction in *T. longicaudatus* is bisexual and hermaphroditic, the latter only in California and other Pacific regions where no males have been recorded.

Young larvae feed on the organic content of the mud and on diatoms, Protozoa, and various small organisms. As they mature, their food and foraging habits vary and a high population may become a serious problem to the rice grower. The rice seed is broadcast in fields flooded to an average depth of 6 inches, which is maintained throughout the season. Young, submerged rice seedlings are delicately rooted and often coated with small gas bubbles. The shrimp dislodge seedlings by chewing leaves and roots and by burrowing in the mud. The dislodged plants often float to the surface, and wind movement collects them in large masses on the rice levees. Such windrows of rice plants on the shoreline do not necessarily indicate shrimp activity, since wind and wave action alone can produce the same effect if the seedbed is prepared improperly and the water is shallow. If shrimp are responsible, however, the uprooted seedlings will generally show some evidence of chewed leaves.

Damage.—To demonstrate chewing damage, rice seeds were placed between two 16-mesh screen disks about 3 inches in diameter in a rice field heavily infested with shrimp. One set of disks and seeds was placed in the rice paddy and protected from shrimp with a 16-mesh screen enclosure. A second set was left open in the infested rice paddy. The unprotected rice plants were chewed until they were considerably retarded or died, as indicated by a 27% seedling reduction in the exposed group of plants. Figure 2 (page 37) shows a set of protected and unprotected plants.

Muddy water is generally a reliable indicator of a shrimp infestation, particularly if only a section of the field is muddy, because shrimp frequently localize in certain rice checks. This localization may be repeated annually in the same checks. Rosenberg (1947) reported that the silt suspension from shrimp movements resulted in poor plant growth.

Chemical tests for control of *T. longicaudatus* with copper sulfate and with DDT were reported by Rosenberg (1947), and with DDT by Portman & Williams (1952) in conjunction with mosquito control. Unsatis-

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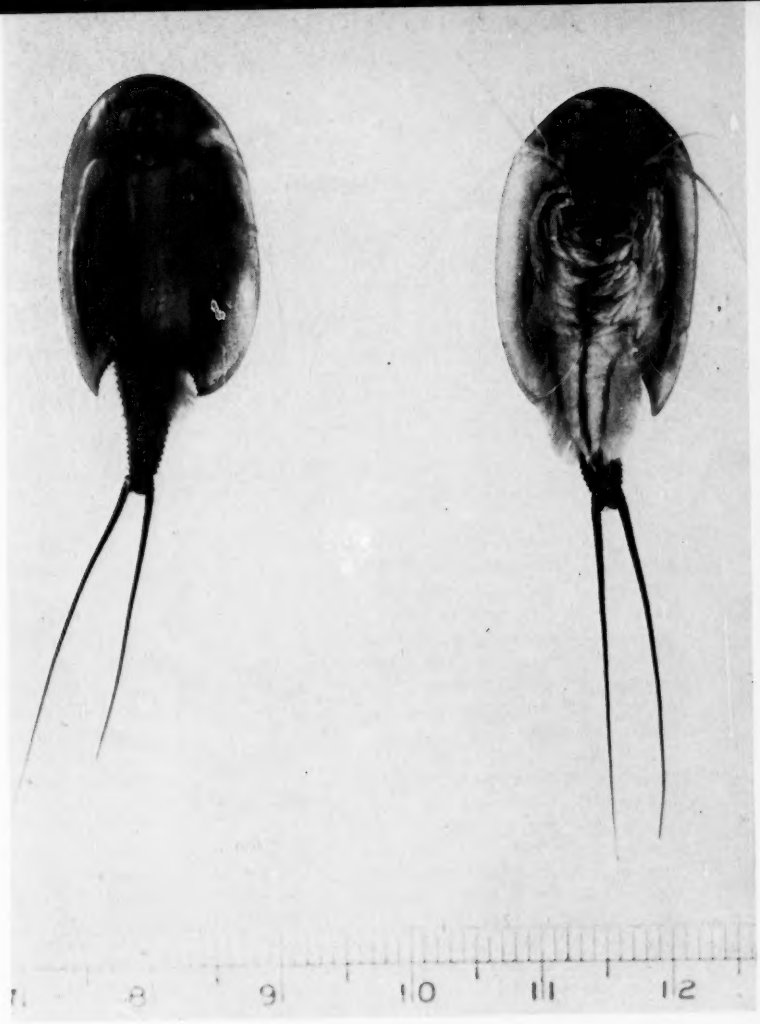

FIG. 1.—*Triops longicaudatus* (Le Conte).

FIG. 2.—Three-week-old rice seedlings from a field heavily infested with shrimps. Left—plants exposed to shrimps. Right—plants protected by a screen.

Table 1.—Results of field test for control of *Triops*, June 17, 1954.

Formulation and Concentration	Active Ingredient/ Acre (lb.)	Per Cent of Shrimp Affected at Hours Shown Following Treatment					
		24		48		72	
		Moribund	Dead	Moribund	Dead	Moribund	Dead
DDT, EC[a] 2.0 lb./gal.	2.0	45	55	0	100	—	—
Dieldrin, EC 1.5 lb./gal.	0.5	98	2	22	78	0	100
Dieldrin, 2%, granules	0.5	57	0	51	49	16	84
Heptachlor, EC 2.0 lb./gal.	0.5	0	0	15	3	28	46
Malathion, 15%, granules	4.0	54	46	0	100	—	—
Copper sulfate, 99%, crystal	10.0	28	13	0	24	0	28
Check	—	0	0	0	0	0	0

[a] EC, Emulsifiable concentrate.

factory control with copper sulfate reported by growers in 1954–55 indicated a possibility of resistance to that chemical. This development, plus outbreaks of the rice leaf miner, *Hydrellia griseola* Fall., and rice seed maggots (chironomid larvae), prompted the present tests. Chemicals were selected for their possible value not only for shrimp control but for control of other rice pests as well. All tests were conducted at the Rice Experiment Station, Biggs, California.

1954 Field Tests.—*Methods.*—The materials and application rates given in table 1 were used in 1/10-acre rice paddys containing water to an average depth of 6 inches. Applications were made with hand sprayers (10 gallons of water per acre) and manual granule dispensers. Two cylindrical cages, made of $\frac{1}{4}$-inch hardware cloth 17 inches high by 18 inches in diameter, were placed one on each end of the plot. About 20 shrimp were placed in each cage, and counts were made 24, 48 and 72 hours after treatment. Percentages of moribund and dead shrimp are reported in table 1. Moribund shrimp were generally at the bottom of the cage, on their backs and exhibiting weak gill movement, though they occasionally swam and sometimes recovered completely. Shrimp were considered dead after gill movements ceased.

Results.—The differences between the test materials (table 1) in speed of action and resultant mortality made it possible to rate them in decreasing order of effectiveness as follows: (1) DDT, (2) malathion, (3) dieldrin, (4) heptachlor, and (5) copper sulfate. The mortality resulting from dieldrin occurred sooner from the emulsion than from the granular form. Mortality from copper sulfate crystals was only 28% at 72 hours, which appeared to substantiate grower reports of poor performance.

1957 Laboratory Tests.—*Methods.*—The following four chemicals were used at dilutions of 1, 2, 4, 20, and 200 parts per million: DDT (2.0 lb. per gal. emulsible concentrate), dieldrin (1.5 lb. per gal.), malathion (4.0 lb. per gal. emulsible concentrate), and copper sulfate (99% crystals placed in saturated aqueous solution). Stock solutions were pipetted into glass jars with 2 liters of rice field water to the desired dilutions. Fifteen field-collected shrimp were divided into 3 replications of 5 per jar for each dilution. A check container of five shrimp accompanied each dilution. The shrimp were kept within the jars by plastic insert screens that allowed the test animals to be brought to the surface for examination. Observations were made periodically, and mortality recorded over a 20-hour period. The criterion for death was lack of gill movement.

Results.—Table 2 shows the time necessary to kill 50% and 100% of the shrimp and the per cent mortality at the end of the test. At the two highest dosages shrimp mortality was complete for all chemicals except DDT and malathion. The chemicals in decreasing order of toxicity at the highest dosage were: (1) DDT and dieldrin, (2) malathion, and (3) copper sulfate. At the lowest dilutions the most effective chemical was copper sulfate.

The surviving treated shrimp were all affected and probably would have died, but the check shrimp were beginning to die from confinement. Mortality in the check was 4% to 10% at 10 hours and 28% to 36% at 20 hours. Mortality at the three lowest dilutions did not always directly correlate with the strength of the dilution. It was felt that more accurate comparisons of toxicants with these laboratory methods would require a greater spread between the chemical dilutions.

1958 Field Trials.—*Methods.*—The methods and plot

Table 2.—Results of laboratory test with chemicals for control of *Triops*, June 6, 1957.

Treatment Concentration		Time (minutes for 50% and 100% mortality) and Per Cent Final Mortality at 20 Hours											
		Copper Sulfate			Dieldrin			DDT			Malathion		
P.p.m.	Lb./Acre	50%	100%	Final	50%	100%	Final	50%	100%	Final	50%	100%	Final
200	278.0	30	64	100	4	6	100	6	8	100	29	37	100
20	27.8	105	156	100	30	40	100	75	—	67	190	—	80
4	5.5	117	192	100	990	—	60	990	—	53	230	—	93
2	2.7	153	197	100	—	—	27	1,020	—	53	232	310	100
1	1.4	147	216	100	—	—	40	—	—	47	285	365	100
Check	—	—	—	28	—	—	36	—	—	36	—	—	36

sizes used in this test were the same as those described in the 1954 trials except that the test shrimp were arranged in three replications of 10, 10, and 20 shrimp per treatment. The 10-shrimp replications were contained in 16-mesh screen cylinders 8 inches in diameter and 18 inches high, with screened bottoms. A total of 33 shrimp were used in the dieldrin treatment. Mortality of the check animals was omitted in table 3 since one replication was killed by chemical drift and another was eaten by predators. Field confinements of this type in other trials have not normally resulted in high mortality.

Table 3.—Results of field test with insecticides for control of *Triops*, June 5, 1958.

Formulation and Concentration	Active Ingredient/ Acre (pounds)	Shrimp Affected at Hours Shown Following Treatment (%)			
		24		48	
		Moribund	Dead	Moribund	Dead
DDT, EC, 2.0 lb./gal.	2.0	97	3	0	100
DDT, 50% granules	4.0	50	30	5	88
Dieldrin, EC, 0.5 lb./gal.	0.5	18	9	52	42
Malathion, EC, 5.0 lb./gal.	2.0	17	78	0	100
Copper sulfate, solution, 99% crystal	10.0	0	100	—	—
Copper sulfate, crystals, 99%	10.0	30	70	0	92
Diazinon, EC, 2.0 lb./gal.	2.0	30	70	0	100
Kepone, EC, 2.0 lb./gal.	2.0	22	68	0	93

Results.—The mortality percentages in table 3 show that the rates of copper sulfate (solution), DDT (emulsible concentrate), malathion (emulsible concentrate), and Diazinon® (emulsible concentrate) (*O, O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) at the dosages selected all gave 100% shrimp control within 48 hours. The other chemicals gave 86% control or better, except dieldrin, which was applied at a considerably lower rate. The high percentage of moribund shrimp in the dieldrin test at the 48-hour interval indicated that more shrimp would have succumbed after an additional 24 hours. The fastest-acting chemical was copper sulfate applied as a solution.

1959 FIELD TESTS.—*Methods.*—The chemicals and rates indicated in table 4 and 5 were applied with hand sprayers and granule dispensers to 1/200-acre sections of a rice field. The treated areas were separated by plastic levees 2 to 3 inches higher than the average water depth of 6 inches. Separate inlets and outlets allowed independent water flow through each treated area. Shrimp were

Table 5.—Results of field test with copper sulfate in solution for control of *Triops*, June 1, 1959.

Active Ingredient/ Acre (lb.)	Shrimp Affected at Hours Shown Following Treatment (%)			
	4		24	
	Moribund	Dead	Moribund	Dead
5.0	27	27	0	97
10.0	0	100	—	—
None	0	0	0	0

field-collected and placed in screened cages like those used in the previous tests. About 50 shrimp were divided into three replications (10-30-10) for each treatment. A new complement of shrimp was used for each of the three series listed in table 4. A second test was made with copper sulfate alone (table 5) in which three replications of shrimp were used (10-10-10). Mortality counts were made in the same manner as for previous tests.

Results.—The various differences noted in table 4 suggest the following ranking of the test chemicals in decreasing order of toxicity: (1) copper sulfate (solution), (2) Sevin® (1-naphthyl methylcarbamate) (3) Diazinon, (4) DDT formulations, (5) malathion, (6) Kepone® (decachloroöctahydro-1, 3, 4-metheno-2*H*-cyclobuta[cd]pentalen-2-one) and (7) copper sulfate (crystals). Sevin was the most effective chemical with short residual properties, and DDT granular formulation A had the longest residual activity. As indicated in table 5, copper sulfate in solution was nearly as effective as one-half the 10-pound rate previously recommended for field use.

DISCUSSION.—These tests indicate not that *Triops longicaudatus* is resistant to copper sulfate but that past failures may be caused by the application practices or the type of crystal formulation used. Control was maximum when the copper sulfate was applied in solution and it appeared that the rate at which it went into solution determined its effectiveness. Since copper sulfate solution is quite corrosive to most application equipment, it should be applied dry, as a dust or at the smallest crystal size available. This chemical caused little shrimp mortality a week after treatment and probably would not be effective when applied before planting or with the seed. The selectivity of copper sulfate and its low toxicity to wild life call for its continued consideration for shrimp control.

Table 4.—Field test of residual activity of chemicals (May 25, 1959) for control of *Triops* introduced into plots along with chemicals and after two intervals.

Formulation and Concentration	Active Ingredient/ Acre (lb.)	Shrimp Affected at Hours Shown Following Treatment (%)													
		Series A—introduced 5/25						Series B—introduced 5/28				Series C—introduced 5/31			
		24		48		72		48		72		24		48	
		Moribund	Dead	Moribund	Dead	Moribund	Dead	Moribund	Dead	Moribund	Dead	Moribund	Dead	Moribund	Dead
DDT, EC, 2.0 lb./gal.	2.0	96	4	28	72	0	100	0	100	—	—	0	0	0	65
DDT, 50%, granule A	2.0	60	7	24	76	0	100	5	95	0	100	10	10	5	85
DDT, 50%, granule B	2.0	62	2	10	79	0	86	20	25	0	65				
Malathion, EC, 5.0 lb./gal	2.0	46	54	40	60	2	98	20	80	0	100	0	0	0	0
Copper sulfate, 99% crystals, solution	10.0	0	100	—	—	—	—	0	35	0	45				
Copper sulfate, 99%, crystals	10.0	2	0	0	24	3	52	0	0	5	5				
Diazinon, EC, 2.0 lb./gal.	2.0	54	46	9	91	0	100	0	100	—	—	5	25	0	45
Kepone, EC, 2.0 lb./gal.	2.0	18	0	33	24	6	89	0	60	0	70				
Sevin, WP, 50%	2.0	70	30	0	100	—	—	0	15	0	30				
Check	—	0	0	0	0	0	3	0	0	0	0	0	0	0	0

DDT at 2 pounds actual per acre is the chemical most widely used for shrimp control. It is being applied as a granular material with the seed, or as a spray soon after the fields are flooded. Where mosquitoes are not resistant to it, it is often used for dual mosquito-shrimp control. Dieldrin and heptachlor at 0.5 pounds per acre (double the concentration used for rice leaf miner control) did not provide adequate control.

Diazinon and malathion gave satisfactory shrimp control in all tests at 2 pounds of active ingredient per acre. Their residual activity was less than that of DDT, but may have been long enough to permit treatment at the time of planting. These chemicals may also be of value in controlling other rice-field pests and should be investigated further, particularly at lower rates. The shrimp mortality obtained from Kepone at 2 pounds of active ingredient per acre varied somewhat in the 2 years it was tested which indicates a necessity for additional study. Sevin was used only in the 1959 field test. Its application at 2 pounds of active ingredient per acre resulted in rapid shrimp control and low residual activity. These attributes plus low vertebrate toxicity make it a logical candidate for future tests.

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Preliminary Study of the Genetics of House Fly (*Musca domestica*) Resistance to Malathion[1]

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ABSTRACT

The genetics of malathion resistance in a strain of house flies (*Musca domestica* L.) was studied by selecting two strains, one susceptible and one resistant to malathion, from a slightly resistant field strain. The method of selection was by collecting eggs from individual females and increasing the population to sufficient numbers to determine their response. After three generations of selection a uniform malathion-resistant strain was obtained. A susceptible strain was obtained in one generation although selection was continued another generation. The parent strain was 1.7 times as resistant as the susceptible strain, while the resistant strain was 62 times as resistant.

All insects used to determine response to malathion were laboratory-reared females which were tested 4 days after peak emergence with solutions made by diluting 95% technical malathion in glass-distilled acetone. Flies were held in recovery jars containing food, at a constant temperature until 24-hour mortality counts were taken.

Strain differences other than malathion resistance were present. The resistant strain required a longer period from egg to adult than the susceptible strain. The males in the susceptible strain emerged significantly earlier than the females. The resistant strain was observed to have a lower fecundity than the parent and susceptible strains. The sex ratio in the selected strains did not differ from a 1:1 ratio.

The F_1 hybrids of reciprocal crosses between the susceptible and resistant strain segregated into 50% susceptible and 50% resistant individuals. The least tenuous explanation for this segregation is that malathion resistance is inherited by two allelic groups exhibiting incomplete duplicate dominance epistasis. The segregation of the F_1 hybrids cannot be explained on the basis of a single gene or independent assortment of multiple genes, as the resistant strain is homogeneous malathion-resistant. The F_2 hybrids did not differ significantly from the F_1 hybrids.

Maternal effects were present in the F_1 hybrids but had disappeared in the F_2 generation. That the F_2 progeny of reciprocal crosses did not differ indicates that no sex-linkage is involved in the inheritance of malathion resistance.

Over the past 14 years, evidence on the development of insecticide resistance has accumulated in more than 40 species of insects of public health importance. The species most frequently studied is the house fly, *Musca domestica* L. The first observation of DDT-tolerance in house flies was reported in 1946 in Sweden and more recently house flies have become resistant to organophosphorus insecticides. The first report was by Keiding (1956) in Denmark. March (1959) has presented the most recent summary of insects resistant to organophosphorus insecticides. Malathion-resistant house flies were reported by March (1959) and Wilson *et al.* (1959). Malathion is widely used for house fly control; therefore, resistance to this chemical is of primary concern.

It appears likely that a high percentage of the species of insects will develop strains showing resistance to insecticides, thus it becomes important to ascertain, in each case, the extent of that resistance. The deciding factor, of course, is the genetic potential for resistance. Although much work has been done on house fly resistance, much more is needed to determine the mode of

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inheritance and number of effective genes. Not only are there differences in the inheritance of resistance to different insecticides but different strains of house flies may have a different mode of inheritance for the same insecticide.

Although the genetics of DDT-resistant house flies has been extensively investigated, comparatively little work has been done on the genetics of house fly resistance to organophosphorus insecticides. Nguy & Busvine (1959) investigated the inheritance of resistance of three strains of house flies: an American strain, resistant to malathion (but not to parathion) and Italian and Danish strains, resistant to parathion (but not to malathion). The results of crossing each strain with a normal colony indicated that both types of resistance are inherited through single dominant gene pairs. American-Italian hybrids were back crossed to normal, the progeny treated with malathion and back crossed again to normal. Treatment with parathion killed all progeny, showing that malathion selection had eliminated all parathion-resistant genes. The two types of genes are therefore considered to be either alleles or closely linked.

MATERIALS AND METHODS.—All flies used for testing and crossing were reared in the laboratory in accordance with the standard procedure of the Chemical Specialties Manufacturers Association, with only the adult diet modified. Adult flies were provided with water and Starlac brand nonfat powdered milk in separate dishes, unless otherwise indicated. The rearing room was maintained at 80° ± 2° F. Relative humidity was not controlled.

The cages used for collecting eggs from individual females were constructed from a 2-inch × 2-inch × ½-inch plastic box. Several small holes were melted in the top by use of a hot wire, and a larger hole was melted in the bottom so the top of a 15-mm. by 45-mm. vial could be inserted. A rack to hold the cages was made by drilling ½-inch diameter holes 1½ inches deep in a 2-inch × 2-inch piece of wood. Absorbent fiber cotton was soaked in the reconstituted milk and placed in the vials. This not only served as a source of nutrition but also as an oviposition site. The milk-soaked cotton was changed during the daily egg collection.

The eggs from single matings were planted in larval medium contained in 6-ounce paper hot-cups. The cups were covered with perforated paper lids and placed in large cages to prevent oviposition by loose flies.

As few pupae were recovered from the small cups, 1-pint food cartons were used for cages. Screen wire was substituted for the bottom. The adult flies were provided with nonfat powdered milk and water in separate ¾-ounce souffle cups. Eggs were usually collected by placing a souffle cup of medium in the cage. The flies were anaesthetized with CO_2 before the cages were opened. The eggs were planted in quart wide-mouth Mason jars or gallon crocks of larval medium, depending on the number collected.

Malathion (95% technical), diluted in glass-distilled acetone, was applied to the test animals topically by use of a microapplicator similar to the one described by Roan & Meada (1953). The syringe was calibrated with mercury and found to deliver 1.13 microliters per stroke.

The strain of house flies from which the malathion-susceptible and -resistant strains were selected was a slightly (1.7 times) malathion-resistant field strain. This strain (JUN) was collected at the Juniata (near Manhattan, Kansas) ranch during the summer of 1958 and had been laboratory-reared for eight generations when selection was begun. Twenty five-day-old female flies were placed in individual cages and eggs were collected. When a strain from a female had increased sufficiently (usually by the F_2 or F_3 generation), it was tested for response to malathion. Offspring from nine of the original 20 were successfully tested. One of these nine strains was selected for susceptibility (JS) and one for resistance (JR) to malathion on the basis of response of relatives; therefore, flies used for breeding were not treated.

Ten 5-day-old females from JS F_3 and 10 from JR F_3 strains were separated and placed in individual cages. Progeny of 5 of the 10 JR females were successfully tested and two (JR-3, JR-8) were saved for further selection. Progeny from 3 of the 10 JS females were successfully tested and one (JSS) was selected for greater susceptibility. Selection of the susceptible strain was discontinued.

Ten 5-day-old females from the JR-8 F_5 strain were placed in individual cages. Progeny from 3 of the 10 JR-8 females were tested. One of the three lines (JRR) showed a homogeneous high resistance to malathion; therefore, it was selected to be the resistant parent in the crosses. Progeny from 2 of the 10 JR-3 females were tested but were discarded because of lack of resistance.

Mass reciprocal crosses were made between JSS and JRR strains. The pupae from the two strains were placed in separate pint food cartons where the adults were allowed to emerge. The adults were sexed every 6 hours and placed in appropriate cages. Eggs were collected in a cup of medium and were planted in gallon crocks of medium.

Four days after peak emergence, females were tested to determine their response to malathion. The test insects were anaesthetized with a minimum of CO_2 to facilitate handling while sexing and treating. The insecticide was applied to the mesosternum, and the flies were placed in pint wide-mouth glass recovery jars, which were covered with cheesecloth. While in the recovery jars, the flies were provided with cellucotton pads saturated with nonfat dry milk dissolved in water. Only 25 flies were placed in each jar and held in a constant temperature box at 80° ± 2° F. until 24-hour mortality counts were made. After readings were taken, the recovery jars were washed in soapy water and rinsed with acetone.

RESULTS.—Nine strains derived from the JUN strain were tested for response to malathion; two gave responses quite different from the parent strain. One (JS) was very susceptible to malathion, while another (JR) was more resistant. These selections were based on low numbers because of low population levels.

The three strains derived from the JS strain were tested to determine their response to malathion. One of these strains (JSS) was slightly more susceptible to malathion. The strains derived from the JR strain responded similarly to it. Two of these strains (JR-3, JR-8) were increased for further selection to malathion resistance.

A uniformly resistant strain (JRR) was selected from the JR-8 strain (Probit regression line presented in figure

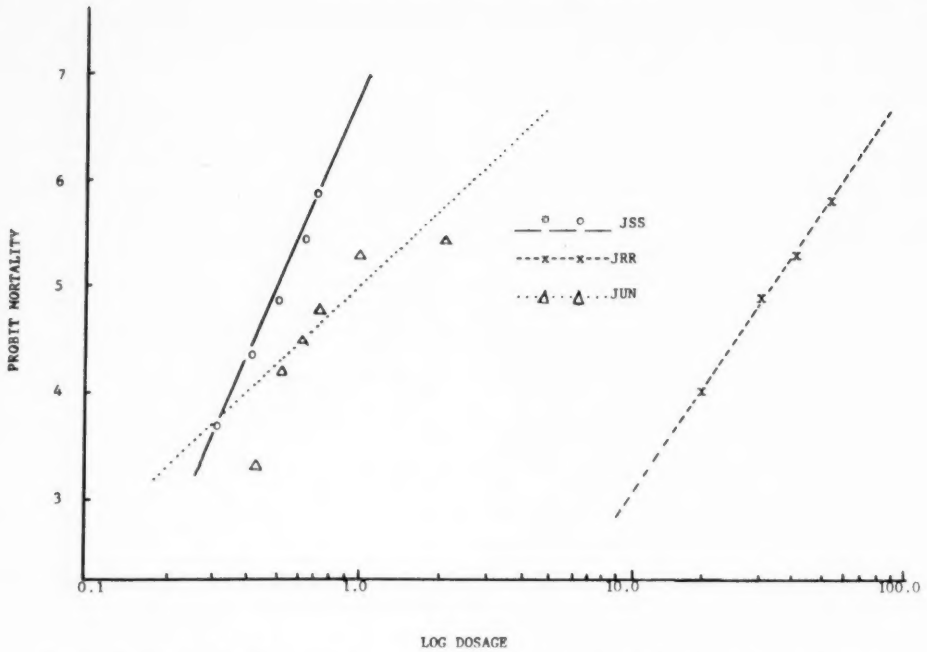

FIG. 1.—Probit regression lines for three strains of house flies treated topically with malathion. Dosages expressed in micrograms per microliter of acetone solution.

1). The other two strains derived from the JR-8 strain were of the same order of resistance as the JR-8 strain. One of the two strains derived from the JR-3 strain was susceptible to malathion while the other responded similarly to the JR-3 strain.

The main difference among the JSS, JRR, and JUN strains is their sensitivity to malathion. The probit regression lines for these three strains of house flies are presented in figure 1. These lines were calculated by the maximum likelihood method as described by Finney (1947). An IBM 650 data-processing machine was used to process the data using a program developed by Sokal (1958).

The LD_{50} values of JSS, JRR, and JUN strains and their 95% fiducial limits are presented in table 1. The LD_{50} values are expressed in micrograms of malathion per gram of body weight. The b values and 95% fiducial limits for each of the strains are also presented in table 1.

The time required for the completion of the life cycle differed in the JRR and in the JSS strains. The JRR strain average life cycle was longer than that for the JSS strain. The males of the JRR strain required an average of 11.0 days to develop from egg to adult; the females, 11.21 days. The JSS strain males averaged 9.77 days and the females, 10.11 days. The data on length of life cycle were analyzed statistically by use of the Kolmogorov Smirnov two-sample test described by Siegel (1956).

Table 1.—LD_{50} (micrograms per gram body weight) and b values for three strains of house flies and F_1 hybrids of reciprocal crosses between the JSS and JRR strain treated with malathion.

STRAIN	LD_{50}	95% FIDUCIAL LIMITS		b	95% FIDUCIAL LIMITS	
		Lower	Upper		Lower	Upper
JUN	36.68	25.90	91.00	2.439	.734	4.145
JSS	23.02	22.29	23.80	6.083	5.337	6.829
JRR	1,427.75	1,317.22	1,546.96	4.292	3.247	5.338
JSS♂×JRR♀	181.50	109.53	267.47	1.125	.737	1.512
JRR♂×JSS♀	123.63	70.43	191.37	1.055	.695	1.415

The sex ratio in the JRR strain was 399 males to 382 females, while the JSS strain was 334 males to 292 females. Chi-square tests for dispersion and heterogeneity were conducted and the difference between sex ratios was nonsignificant ($\chi^2 = 0.714$).

The F_1 progeny of reciprocal crosses between JSS and JRR strains were tested to determine their sensitivity to malathion. The probit regression lines for these reciprocals are presented in figure 2. The calculated LD_{50} and b values for the crosses are presented in table 1. A paired-replicates test for differences between the F_1 generation of reciprocal crosses was calculated according to methods described by Wilcoxon (1949).

The F_2 progeny of the reciprocal crosses were tested using the same doses as were used in the F_1 generation. The probit log-dosage transformation was not graphed as the F_2 response to malathion is similar to the F_1 response. A paired-replicates test for differences between the F_2 reciprocal crosses was calculated. The same test for differences between the F_1 and F_2 generations is presented in table 2.

The data presented in figure 2 indicate that doses between 3 and 7 micrograms of malathion per fly will kill all the susceptible flies and none of the resistant flies.

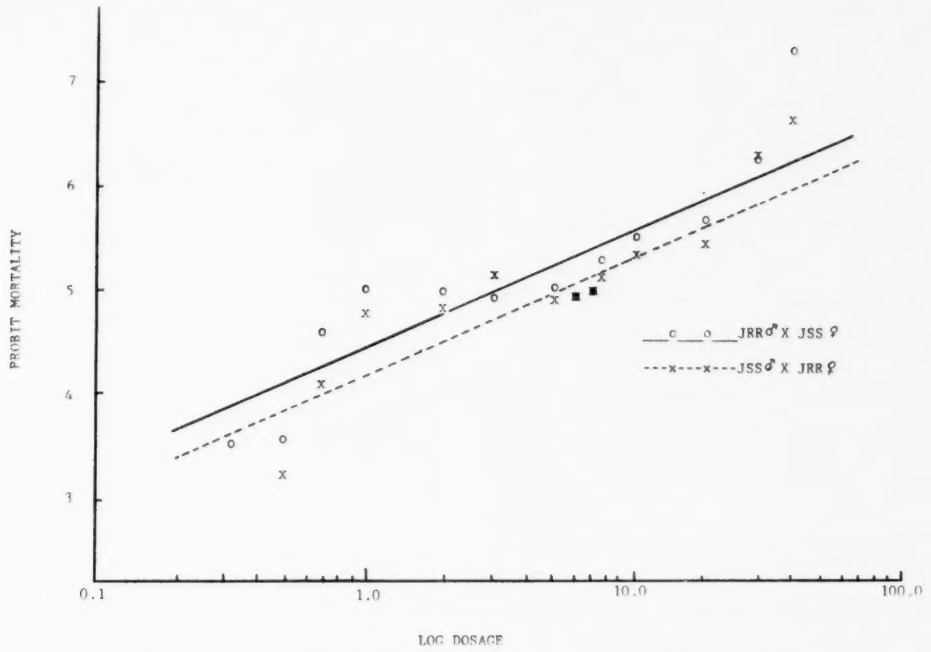

FIG. 2.—Probit regression lines for reciprocal crosses between the JRR and JSS strains of house flies. Dosage expressed in micrograms per microliter of acetone solution.

Table 2.—Paired-replicates test for differences in response to malathion among F_1 and F_2 generations of crosses between the JSS and JRR strains of house flies.

Dose (μG./μL.)	Per Cent Mortality F_1	Per Cent Mortality F_2	Difference	Rank	Lower Rank Total of Same Sign
1.0	46.5	46.2	+ .3	+ 1.5	
2.0	47.0	51.7	− 4.7	− 8.0	8.0
3.0	52.0	51.7	+ .3	+ 1.5	
5.0	48.5	52.7	− 4.2	− 6.0	6.0
6.0	47.5	44.7	+ 2.8	+ 4.5	
			+ 2.8		
7.0	51.5	48.7	+ 9.5	+ 4.5	
8.0	57.5	48.0	+14.5	+10.0	
10.0	66.5	52.0	+ 2.0	+11.0	
20.0	72.0	70.0	+ 7.7	+ 3.0	
30.0	91.0	83.3	+ 4.3	+ 9.0	
40.0	97.0	92.7		+ 7.0	
				Total	14.0 ns

ns $T_{.05}=11$ (when $N=11$)

DISCUSSION.—About one-third of the individuals in the parent strain were resistant to malathion. Selection by testing progeny of individual females would have been impractical if the frequency had been much lower. However, this method of selection proved to be very effective as strains resistant and susceptible to malathion were successfully selected.

Selection for susceptibility to malathion was discontinued after two generations. The JS and JSS strains were as susceptible as an insecticide-susceptible laboratory strain (KUN); therefore, it was feared any further selection would result in loss of vigor. Selection of the second generation was not necessary, as the JS and JSS strains responded similarly.

Three generations of selection were required to produce a uniformly resistant strain. The frequency of resistant and susceptible individuals in the parent strain could account for the difference in the number of generations required to select the two strains. An increase in malathion resistance was obtained in selection of the first generation; however, there was little increase caused by selection of the second generation. The largest increase was obtained in the third generation, when the JRR strain was selected. The JRR strain was selected from the JR-8 strain although it showed a greater sensitivity to malathion than the JR-3 strain.

The JUN strain was 1.7 times as resistant as the JSS strain, whereas the JRR strain was 62 times as resistant (based on LD_{50}). The differences may be considered significant since the 95% fiducial limits on the LD_{50}'s did not overlap. The JRR and JSS strains showed a more homogeneous response than the JUN strain did, as indicated by the b values. The b values for the JSS and JRR strains were not significantly different, while the JSS strain differed significantly from the JUN strain. This was caused, probably, by the fact that the JSS strain was relatively homogeneous susceptible population, whereas the JUN strain was a mixture of resistant and susceptible

individuals, as evidenced by the wide fiducial limits for the b value of JUN. The b value for JRR, though not significantly different from that for JUN, may have differed if higher doses had been included because the regression lines plotted in figure 1 indicate that the JRR strain was a relatively homogeneous resistant population while the JUN strain was less homogeneous.

Data were recorded on time required from egg to adult emergence for each of the selected strains. The JRR strain required an average of 1.16 days longer than the JSS strain. The difference was significant beyond the 0.001 level of probability. The JRR strain was much more heterogeneous in its emergence. These results are similar to those obtained by Pimentel *et. al.* (1951), although they worked with a DDT-resistant strain of house flies.

The males in the JSS strain emerged significantly ($P < .01$) earlier than the females, but the length of life cycle of males and females in the JRR strain did not differ significantly. The sex ratios for both strains were determined but neither differed significantly from a 1:1 ratio.

Although no data were taken on fecundity, the JRR strain usually laid fewer eggs and produced fewer offspring than either the JUN or JSS strains. This condition probably could have been avoided if fecundity had been a selection character. Since selection for high fecundity might have limited selection for resistance, it was not considered.

Reciprocal crosses between the JSS and JRR strains gave F_1 hybrids of which about 50% were susceptible and about 50% were resistant. This phenomenon cannot readily be explained on the basis of a single gene or independent assortment of multiple genes because the JRR strain is homogeneously malathion-resistant. Although there are several possible explanations of the mode of inheritance, the authors feel that the most plausible explanation is incomplete duplicate dominance epistasis. Two basic assumptions are made in this explanation: (1) two sets of alleles affect the inheritance of malathion resistance and (2) an individual containing any two genes for resistance, irrespective of locus, is phenotypically resistant. One of the possible resistance genes will be designated as A and its allele a, the other B and its allele b.

The susceptible strain, under this theory, would have the genotype aabb, as no resistant individuals were detected. The resistant strain could contain individuals with the following genotypes AABB, AABb, and AAbb and all would be malathion-resistant. Since this is a notation for convenience, Ab and aB are interchangeable. Crossing the two strains would result in F_1 hybrids having the genotypes AaBb and Aabb. The genotype Aabb would be susceptible as it contains only one gene for resistance. The ratio of genotype AaBb to Aabb would would be 1:1 if the frequency of the b allele in the resistant strain were $\frac{1}{2}$.

Although it is difficult to determine which dose will kill all susceptible individuals and no resistant individuals, doses between 3 and 7 micrograms per fly are probably near the separation dose. When only doses within this range were considered and the mortality percentages of the F_1 hybrids of reciprocal crosses were averaged, the average per cent mortality was 49.81. The ratio of susceptible and resistant individuals in the F_1 did not differ significantly ($\chi^2 = 0.73$) from the 1:1 theoretical ratio. The F_1 hybrids would have been called intermediate to the parental strains if these mid-dosages had not been well sampled.

The selection method used in these experiments could explain how the frequency of the b allele in the JRR strain could be $\frac{1}{2}$. The JRR strain was derived from one female and, as no more than 2% of the females mate more than once (Zingrone *et al.* 1959), it is safe to assume that this strain came from a single pair of house flies. The proposed frequency would occur if the mating were either of the following genotypic matings: AABb×AABb or AABB×AAbb.

The average mortality of the F_2 generation within the dose range of 3 to 7 micrograms per fly was 51%. The F_2 would segregate according to a 31 resistant to 33 susceptible ratio if there were epistasis but no linkage. The experimental data fit this ratio ($\chi^2 = 0.052$). However, if the two proposed loci affecting resistance were on the same chromosome and so closely linked as to make crossing over negligible, the expected ratio would be 1:1. The observed segregation ratio did not differ significantly ($\chi^2 = 0.285$) from a 1:1 ratio, nor was there evidence of significant heterogeneity between the F_1 and F_2 ($\chi^2 = 0.992$). Since the expected ratios for independent segregation and perfect linkage are so similar, it could not be determined whether the two proposed sets of alleles are linked.

The question of linkage is an important one, for if the two allelic groups were closely linked three different conclusions on the mode of inheritance of malathion resistance could be drawn, depending on the frequency of the genotypes in the resistant strain. A resistant strain containing only the genotype AB/AB, when crossed with the susceptible strain, would suggest that resistance is inherited through one dominant gene as suggested by Nguy & Busvine (1959). A mixture of the genotypes AB/AB, AB/Ab and Ab/Ab would lead to the conclusion drawn in this paper. A resistant strain containing the genotype Ab/Ab, when crossed with the susceptible strain, would suggest that resistance is inherited by a recessive gene.

Although the fiducial limits for the LD_{50}'s overlap, the F_1 hybrids of reciprocal crosses differed significantly ($P < .02$) when tested by use of the paired-replicates test. The paired-replicates test is probably the better test as the regression lines were not good fits. The offspring from the JRR ♂×JSS ♀ cross were more susceptible than the offspring from the JSS ♂×JRR ♀ cross. Pimentel *et al.* (1954) and Johnston *et al.* (1954) found similar maternal effects when working with two different DDT-resistant strains. Maternal effects had disappeared by the F_2 generation as there was no significant difference between reciprocal crosses.

That the F_2 reciprocal crosses were not different indicates that the genes are not sex linked. The type of segregation expected, if sex linkage is present, is well illustrated by Dickson's work (1941) on cyanide-resistant California red scale. Only females were tested and he found that the percentage surviving in the F_2 was 38.2%

for the R ♂ × S ♀ cross and 17.9% for the S ♂ × R ♀ cross.

A second set of reciprocal crosses was made to demonstrate the repeatability of the experiment. A paired-replicate test revealed that the two pairs of crosses did not differ significantly, thereby indicating that the F_1 generation studied was representative.

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Effect of Some Variations in Formulation or Application Procedure on Control of the Imported Fire Ant with Granular Heptachlor[1]

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ABSTRACT

The results of field tests against the imported fire ant (*Solenopsis saevissima* v. *richteri* Forel) with various application rates, formulations and split applications of granulated heptachlor showed that: (1) there was no appreciable difference in the immediate kill obtained with bulk granular applications at rates of 5 pounds of 20% heptachlor versus 20 pounds of 5%, and 10 pounds of 20% heptachlor versus 20 pounds of 10%; (2) formulations with calcined attapulgite granules gave slower initial kill than ones with regular granules but there was not an appreciable difference after 1 year; (3) formulations with a large mesh size granule (8 to 20) gave slightly slower initial kill than a fine mesh granule (30 to 60), but after 2 months there was no appreciable difference and at the end of 1 year they showed a slightly greater kill; (4) two applications of heptachlor at dosages of ¼ or ½ pound per acre put out at intervals of 3 or 6 months were completely effective in controlling imported fire ants. Two applications of one-eighth pound per acre gave a very high degree of control.

The published research work on control of the imported fire ant, *Solenopsis saevissima* v. *richteri* Forel, prior to 1957 has been reviewed by Blake *et al.* (1959). This work had shown that good control of imported fire ants could be obtained with chlordane, when used as a broadcast treatment. Unpublished work by the U.S. Department of Agriculture (Coarsey 1952) had shown that aldrin, heptachlor and dieldrin would also give control. Green & Hutchins (1958) reported that dieldrin and heptachlor, pound for pound, gave similar results when applied as sprays, granular, or fertilizer mixtures. Blake *et al.* (1959) obtained highly effective control with granular formulations of 2 pounds of actual dieldrin and heptachlor and 4 pounds of chlordane per acre. In one test granular dieldrin gave a better residual than a dieldrin emulsion spray. Lower dosages gave good control for 1 year after treatment. An insecticide-fertilizer mixture was as effective as the insecticides applied alone.

After the imported fire ant eradication program was initiated by an act of the United States Congress in 1957, the preceding information was used as the basis for establishing the standard treatments which were 2 pounds of heptachlor or dieldrin as granular formulation.[2] They were applied at the rate of 20 pounds of 10% granules per acre as a surface treatment. The formulation consisted of 18- to 60-mesh attapulgite granules, a solvent (methylated napthalene), deactivator and insecticide. At this time there was little or no information available regarding the effect on imported fire ant control of variations in the formulation, bulk application rates and low dosage applications.

This paper presents the results of tests conducted to determine the effect on control of imported fire ants by granular heptachlor when (1) various mesh sizes of regular and calcined attapulgite granules were used, (2) the

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[2] On the basis of this and subsequent work the recommended dosage is now two applications of ¼ pound of heptachlor, each applied as 12½ pounds of 2% granular material.

Table 1.—Effect of different bulk application rates on the control of imported fire ants with granular heptachlor when the actual insecticide dosage per acre remained constant.

Granule Application Rate (lb./acre)	Dosage (lbs./acre)	Average Pre-treatment Count	Per Cent Reduction in Active Colonies After Following Weeks								
			1	2	4	8	16	26	32	52	78
5	1	40	48	67	88	99	93	93	89	63	76
20	1	36	55	67	90	99	98	98	96	94	96
10	2	41	48	79	90	100	100	100	99	100	98
20[a]	2	34	33	40	82	97	97	97	81	63	87
Check	—	31	11	15	18	34	26	13	6	2	2

[a] Average of 2 replications. All others are the average of 3 replications.

application rate of total granular formulation was varied and (3) two low dosage applications were made at 3- or 6-month intervals.

TEST I.—In May 1958 an experiment was initiated to determine if the control obtained with granular heptachlor would be altered when the pounds of total formulation applied was varied while the pounds of actual insecticide per acre was kept constant. In other words, this test would help to determine the actual number of granules of insecticide or coverage necessary to obtain imported fire ant control. Application rates of 10 pounds of 20% heptachlor granules per acre and 20 pounds of 10% (2 pounds per acre of active ingredient) were compared as well as rates of 20 pounds of 5% material and 5 pounds of 20% (1 pound per acre of active ingredient). The granule used was 18- to 60-mesh attapulgite clay. In both tests enough untreated granules were added to the 20% material to permit application of the formulations at the rate of 20 pounds per acre. The applications were made with a Buffalo turbine blower. Two of the fields treated were open pastures and the third was a pecan grove and pasture. The average pretreatment counts of active imported fire ant mounds per plot, and the per cent reduction at various intervals after treatment, are recorded in table 1.

After 2 months the control obtained with the various treatments was about equal, which indicates that the bulk application rate had no apparent effect on the immediate control obtained. Eight months after treatment a noticeable decline in control was noted with the 20 pound-rate of 10% heptachlor and the 5-pound-rate of 20% heptachlor. This was caused principally by a reinfestation in one plot of each set. The field in which this series of replications was run did not receive any rainfall for about 3 weeks after treatment. Control lagged during these 3 weeks but climbed rapidly after the first rain. It is now known that heptachlor can be lost from intact granules which are exposed to hot, dry conditions (Barthel *et al.* 1959). Since these conditions did occur during the first 3 weeks after treatment there was probably a considerable loss of insecticide. This theoretical rapid loss of heptachlor could account for the early reinfestation of these plots.

TEST II.—A comparison was made of the effect of granule mesh size range and water breakdown properties of granular attapulgite heptachlor formulations on control of imported fire ants. Four different mesh sizes (8 to 20, 16 to 30, 20 to 40, 30 to 60) of the regular and calcined granules were compared. The latter type of granule does not break down in water. The heptachlor content of each formulation was 2.5%. The granules were applied to small plots ($\frac{3}{4}$ to 1 acre) with an 8-ft. Gandy fertilizer distributor at the rate of 20 pounds per acre or 0.5 pounds per acre of actual heptachlor. The plots were located in a Bahia grass pasture. The tests were run in triplicate. The treatments were made in July 1958. The rainfall during the first 8 weeks after treatment was very high (20.5 inches).

Table 2.—The effect of variations in granule mesh size and water breakdown properties of heptachlor granular formulations on their control of imported fire ants in field plots.[a]

Granule Mesh Size (range)	Average Pre-treatment Count	Per Cent Reduction in Active Colonies After Following Weeks							
		1	2	4	8	16	32	52	78
Regular Granules—AARVM									
8–20	22	61	74	82	97	98	95	100	91
16–30	14	60	86	86	100	93	93	95	97
20–40	13	59	80	95	100	100	92	97	98
30–60	18	69	87	98	100	94	91	94	81
Calcined Granules—AALVM									
8–20	17	40	48	71	92	94	90	100	98
16–30	20	42	63	85	97	100	95	95	92
20–40	15	36	64	84	100	100	95	95	89
30–60	18	38	72	91	94	96	92	89	89
Check	15	17	30	9	20	0	0	16	0

[a] Average of 3 replications; heptachlor dosage 0.5 pound per acre.

The results based on the per cent reduction in active imported fire ant colonies before and after treatment (see table 2) show that during the first month after treatment the control obtained with the formulations of the coarser mesh granules (8 to 20) was slightly slower than with the finer mesh granules (30 to 60). This was also true with the calcined granules when compared with the regular granules. After 4 months there was no appreciable difference in the control obtained with any of the formulations. The 1-year counts showed a definite trend toward better control with the coarser granular formulations. The same trend was evident after $1\frac{1}{2}$ years except for the 8 to 20 mesh AARVM granules. The decrease in control with this formulation was caused principally by a slight reinfestation in one plot. It is noteworthy that 4 of 5 of the colonies present were either on the edge or in the treatment overlap area where there might possibly have been a skip.

TEST III.—Three series of test plots were set up to evaluate the effectiveness of two treatments of $\frac{1}{2}$, $\frac{1}{4}$ or $\frac{1}{8}$ pounds of heptachlor per acre for controlling imported

fire ants. One series of plots was retreated after 3 months, a second series after 6 months and a third series received only one treatment. The test plots were located in a Bahia grass pasture and were about 2 acres in size (200 ft.×400 ft.). The granular insecticide formulations (1.25%, 2.5% and 5.0%) were applied with a 20-ft. Gandy fertilizer distributor at the rate of 10 pounds of granules per acre. The first application was made in January 1959. The first series was retreated in April and the second series in August. All tests were run in triplicate. Pretreatment and posttreatment counts of active imported fire ant colonies and the average per cent reduction after treatment are recorded in table 3.

After 1 year 100% control was obtained on all plots receiving two treatments of $\frac{1}{4}$ or $\frac{1}{2}$ pound of heptachlor per acre. Four of the $\frac{1}{8}$ pound per acre retreatment plots were free of imported fire ants; the remaining 2 plots had 2 and 1 active mounds, respectively, directly on the edge of the plot. In the series of plots which received only one treatment, the $\frac{1}{2}$-pound per acre dosage gave 99% control, the $\frac{1}{4}$-pound per acre, 100% and the $\frac{1}{8}$-pound per acre, 79%.

Table 3.—Results of field tests against imported fire ants with two low-dosage treatments of heptachlor applied at intervals of 3 or 6 months.[a]

Dosage (lb./acre)	No. of Treatments	Interval Between Treatments (months)[b]	Average Pretreatment Count	Per Cent Reduction in Active Mounds After Months Shown			
				3	6	9	12
0.125	1	—	24	18	63	82	79
	2	3	45	24	94	98	99
	2	6	53	36	82	99	99
0.25	1	—	34	51	88	98	100
	2	3	32	46	99	100	100
	2	6	49	47	97	100	100
0.5	1	—	32	60	96	98	99
	2	3	31	56	100	100	100
	2	6	47	49	98	100	100
Check	—	—	48	7	13	31	34

[a] Average of 3 tests.

[b] Initial application made on January 26, 1959, and the retreatments on April 16, 1959, and August 7–8, 1959.

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Control of the Cabbage Looper with a Nuclear Polyhedrosis Virus Disease[1]

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ABSTRACT

A nuclear polyhedrosis virus disease was found to be effective in controlling the cabbage looper (*Trichoplusia ni* (Hbn.)) on cabbage, cauliflower, and lettuce in southern California. A virus concentrate of 5.5 billion polyhedra per milliliter was used in 1957 at rates of 1, 5, and 10 ml. per gallon. In April the time required from exposure of hatching larvae until most of them died was 20 days. In September cabbage loopers were not successfully controlled nor was leaf damage prevented after two applications of virus were made to half-grown cauliflower when loopers had become large enough to cause some leaf damage before dying. However, virus applications on small plants soon after thinning, when loopers were small or in the egg stage, gave much better control. Three applications protected a cauliflower field from plant thinning until harvest time.

In southern California the cabbage looper (*Trichoplusia ni* (Hbn.)) is an important pest of cabbage, cauliflower, and other cruciferous crops, as well as lettuce, celery, and peppers. The experiments discussed in this paper were carried on at Whittier in 1957 for the purpose of determining the effect of a nuclear polyhedrosis virus disease on the cabbage looper.

This disease has been known in the United States for many years. Hall (1957) reported its effectiveness against the cabbage looper on lettuce in California. He also reviewed the literature from the first records of its occurrence and use up to 1957. His work on lettuce indicated that mortality of both large and small cabbage loopers could be expected in about 9 days after exposure at moderate temperatures. He applied infectious virus material at the rate of 12 gallons per acre at three spray concentrations: 1, 5, and 10 million polyhedra per milliliter. His highest dosage was almost 100% effective. He concluded that thorough plant coverage was essential.

McEwen & Hervey (1958) obtained effective control with this virus disease on cabbage, cauliflower, and broccoli in New York. They based the virulence of infectious spray material on the number of dead diseased loopers it contained. Hemacytometer counts indicated that their lowest spray dilution contained 0.6 million, and the highest 7.6 million polyhedra per milliliter. Spray was applied at the rate of 30 gallons per acre.

MATERIALS AND METHODS.—The virus concentrate used in the tests reported in this paper was obtained from cabbage loopers that had died of virus disease.

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After a period of putrefaction, the material was diluted, filtered, and the polyhedra per milliliter adjusted to 5.5 billion. This concentrate was used in all experiments except as noted in table 3. Spray was directed from both sides and from above the plants with a hand-operated sprayer at 50 to 85 gallons per acre. Four ounces of a wetting agent (Colloidal X-77) were used per 100 gallons of spray. Cabbage and cauliflower leaves have a natural waxy coating and are difficult to wet. Preliminary experiments revealed that virus concentrate spray could not be applied properly without using a suitable amount of an effective wetting agent.

Field plots were 3 to 4 rows wide and 40 to 75 feet long, depending on the shape and size of each field. Five to six replicates were used in each case. The per cent of control was determined by counting surviving looper larvae on 10 plants per plot, except as noted. Leaf damage indices were based on zero for no damage, and one to four for different degrees of damage on 10 plants per plot.

LABORATORY TESTS.—The first experiment was arranged to determine the time from exposure of young or newly hatched larvae to virus disease until death, and to explore the possibility of applying this material to field plants. Because of a temporary scarcity of cabbage loopers in the coastal area of Southern California during April, when the first tests were begun, a method of artificial looper infestation was used. Eggs were obtained from moths emerging from looper caterpillars collected near Indio. These eggs were laid on blotting paper, and were dipped in 10% formaldehyde for 45 minutes, and washed in water for 1 hour before use. Cabbage plants in field plots were sprayed with virus, and, as soon as the spray had dried, leaves from treated plants were collected and put in quart-sized food cartons. Twenty-five eggs, ready to hatch, were then placed in each of six cartons per treatment. The cartons were stored at outdoor temperatures for observation. Mean temperature for this period was 61.7° F. Results are presented in table 1.

Table 1.—Reduction of healthy cabbage loopers after placement of 24 eggs ready to hatch on cabbage leaves from a field sprayed with polyhedrosis virus at 85 gallons per acre.

Virus Concentrate per Gallon (ml.)	Number of Loopers After Indicated Days					Control After 22 Days (%)
	4	10	15	19	22	
0	21	21	19	19	19	—
1	21	21	21	16	13	32
5	21	17	14	0.3	0	100
10	19	14	12	2	0	100
L.S.D. at 5% level	N.S.	N.S.	7	5.5	6.3	33

Only moribund or dead larvae were considered to be affected by the disease and were removed at each observation. Fresh leaves from the treated plots were supplied when needed as food for surviving caterpillars.

FIELD TESTS.—The next step in evaluating this virus as a control was to artificially infest plants in the field with looper eggs. As a substitute for natural infestation, eggs laid on red blotting paper in the laboratory were pinned to cabbage leaves. Twelve to fifteen eggs were pinned to each of 20 plants per plot after the virus spray had been applied on April 17. Daily mean temperatures during the period of this experiment ranged from a low of 52° F. on April 20, to a high of 70° on April 28. The only precipitation occurred on April 17, 18, 20, and 22, and totaled 1.47 inches. Effectiveness was based on the number of surviving looper larvae at 8, 12, and 21 days after the eggs had hatched on April 18 (table 2).

Table 2.—Reduction of healthy cabbage loopers per plant after placement of 12 to 15 eggs ready to hatch on 20 cabbage plants per plot sprayed with polyhedrosis virus at 50 gallons per acre.

Virus Concentrate per Gallon (ml.)	No. of Loopers After Indicated Days			Control After 21 Days (%)
	8	12	21	
0	5.4	5.4	3.5	—
1	5.4	3.6	.3	90
5	4.7	1.9	.2	93
10	4.9	1.2	.2	92
L.S.D. at 5% level	N.S.	2.2	1.2	N.S.

Experimental use of this virus disease was continued in September when temperatures were fairly high and the cabbage looper was numerous on field plantings of crops it usually attacks. Daily maximum temperatures in September ranged from 77° to 90° F.; daily mean, from 68° to 77° F.; and the monthly average was 72.2°. There was no precipitation in September.

The first experimental field was of cauliflower already half-grown and heavily infested by loopers in all stages of development (table 3). Two applications of virus concentrate, 11 days apart, killed most of the loopers; but the long disease incubation period permitted large caterpillars to continue feeding after the first application. After 21 days, it was evident that much leaf damage had occurred and that about one looper per plant had survived the treatment. Apparently the slow action of this virus disease makes it unsuitable as a control on nearly mature plants and after cabbage loopers have grown large enough to feed extensively before succumbing to the disease.

Table 3.—Reduction of healthy cabbage loopers per plant after first treatment when two applications of polyhedrosis virus were made on half-grown cauliflower at 50 and 75 gallons per acre.[a]

Virus Concentrate per Gallon (ml.)	No. of Loopers After First Treatment		Control After 21 Days (%)
	11 Days	21 Days	
0	1.4	4.5	—
1	1.6	1.6	65
5	1.0	1.1	75
10	1.0	1.0	78
L.S.D. at 5% level	N.S.	1.2	N.S.

[a] Virus concentrate contained 4.5 billion polyhedra per ml. Sept. 5, and 5.5 billion polyhedra per ml. Sept. 16.

Failure to control the cabbage looper successfully in the type of experiment just described led to further field

trials. In the next three experimental fields, two of cauliflower and one of lettuce, virus spray was applied soon after plant thinning. The small plants could be more thoroughly covered, and observations revealed that loopers were all small or in the egg stage. Introduction of the virus disease early in crop development also appeared to be the best opportunity for establishing field contamination as a means of crop protection.

Table 4.—Reduction of healthy cabbage loopers per plant after application of polyhedrosis virus spray at 50 gallons per acre in three fields soon after plant thinning.[a]

Virus Concentrate per Gallon (ml.)	No. of Loopers per Plant	Control (%)
Cauliflower: 9 Days after Application		
0	4.5	—
1	1.9	58
5	1.5	63
10	1.0	78
L.S.D. at 5% level	1.9	N.S.
Cauliflower: 12 Days after Application		
0	13.5	—
1	2.8	80
5	0.7	95
10	.9	93
L.S.D. at 5% level	3.1	N.S.
Lettuce: 17 Days after Application		
0	0.9	—
1	.07	92
5	.02	98
10	.03	96
L.S.D. at 5% level	N.S.	N.S.

[a] A second application at 75 gallons per acre was made on cauliflower after 12 days and on lettuce after 7 days.

In the field (table 4), work was discontinued after 9 days because the cooperator refused to leave the checks untreated. At this time the disease probably had not attained its full effect. In the other cauliflower field the disease had attained its full effect in 12 days when the counts were made. The lettuce field received two applications 7 days apart because of more rapid plant growth, and counts were made 17 days after the first application.

At this point, virus control experiments had dealt only with the early period of plant development in cabbage, cauliflower, and lettuce fields. Control had been good when small loopers were exposed because the small amount of feeding during the comparatively long virus incubation period was not noticeable in terms of crop damage. Likewise, by having the disease well established on the plants, additional loopers hatching from eggs became infected and died while still small. Since the virus disease was effective in protecting small host plants, the next step was its experimental use for the entire period of crop development. Therefore, in a field of cauliflower, three treatments were made 9 and 12 days apart, beginning soon after plant thinning. Observations were made as shown in table 5, and continued until harvest time. On September 20, 9 days following the first disease application, maximum effectiveness had not been reached. On October 3, 22 days after the first application, and 13 days following the second, looper control was greater than 90%, with the 5- and 10-ml. concentrations. Effective control was still apparent after the third application, and at 65 days following the first treatment. Temperatures were about the same in October as in September. A total precipitation of 1.51 inches occurred from October 11 to 31. There was no rain in November until the 15th, but the temperatures were higher than during October, 80° to 95° F.

Discussion.—This disease of the cabbage looper appears to be quite virulent under a wide variety of weather conditions. In experiments conducted during April, when mean temperatures ranged below and a few degrees above 60° F., the disease was as effective as it was during September when temperatures were much higher.

Virus activity was slowed up considerably at lower temperatures, but laboratory-rearing experience led to the conclusion that conditions which induce moths to emerge and oviposit will also permit the virus to develop.

It is unlikely that relative humidity ever becomes low enough in irrigated fields in which cruciferous crops are grown in Southern California to affect virulence.

Dosages of 1, 5, and 10 ml. of virus concentrate per gallon of spray were used without knowing whether they were in the correct range. In general, 5 and 10 ml. per gallon of a virus concentrate, containing 5.5×10^9 polyhedra per milliliter, were more effective than 1 ml., but 10 ml. gave very little, if any, greater control than 5. It is probable that less than 5 ml. of the virus concentration

Table 5.—Reduction of healthy cabbage loopers per plant and leaf damage index in a cauliflower field 9 to 65 days after the application of polyhedrosis virus spray at 50 gallons per acre soon after thinning on September 11.[a]

	9 Days		22 Days		35 Days				65 Days			
					Larvae		Leaf Injury		Larvae		Leaf Injury	
Virus Concentrate per Gallon (ml.)	No. per Plant	Control (%)	No. per Plant	Control (%)	No. per Plant	Control (%)	Index	Reduction (%)	No. per Plant	Control (%)	Index[b]	Reduction (%)
0	1.30	—	2.10	—	1.24	—	111	—	5.80	—	104	—
1	.94	28	.48	77	.08	94	11	90	.10	98	13	89
5	.38	71	.16	92	.04	97	2	98	.16	97	23	78
10	.42	68	.12	94	.08	94	5	96	.12	98	26	75
L.S.D. at 5% level	.61	n.s.	.52	n.s.	.17	n.s.	9.7	n.s.	.33	n.s.	24	n.s.

[a] Additional applications of 60 and 75 gallons per acre were made on September 20 and October 2, respectively.
[b] After 65 days leaf damage was difficult to index because of an invasion of salt-marsh caterpillars (*Estigmene acrea* (Drury)).

used in these tests would be enough to obtain control of the cabbage looper.

The effect of more than one application of virus concentrate was not demonstrated in these experiments. One application on small plants transmitted the disease to a high percentage of loopers on the plants; additional applications were made as shown in table 5 to protect the plants as they grew. Two or more applications are usually required to protect most crops attacked by the cabbage looper because of the necessity of covering additional leaf area caused by plant growth.

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Further Studies of Integrated Control Methods against the Egyptian Alfalfa Weevil in California[1,2,3]

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ABSTRACT

The alfalfa weevil, *Hypera postica* (Gyll.), and the Egyptian alfalfa weevil, *H. brunneipennis* (Boheman), are minor pests on alfalfa in California. Chemicals of indiscriminate toxicity directed against these two minor pests could initiate more serious pest problems in alfalfa. Several insecticides were evaluated for their effect on the Egyptian alfalfa weevil, on *Bathyplectes curculionis* (Thoms.), a parasite of the two weevils, and on other parasites and predators of field crop pests. Heptachlor at low dosages of 2 to 2.4 ounces per acre gave effective control of the two weevil larvae but had little effect on *B. curculionis*. At these low dosages heptachlor had little effect on *Hippodamia convergens* G.-M. and *Orius tristicolor* (White) but had a moderate effect on *Trioxys utilis* Mues., a parasite of the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)), and on *Chrysopa* spp., and *Nabis ferus* (Linn.). Demeton which is used to control aphid pests in alfalfa hay and seed in California, had little or no effect on the Egyptian alfalfa weevil larvae. It had a moderate effect on the weevil parasite *B. curculionis* and on *N. ferus* and *O. tristicolor* and it had a slightly greater effect on *T. utilis* and *H. convergens*. The biotic insecticide, *Bacillus thuringiensis* Berliner, had little, if any, effect on the Egyptian alfalfa weevil larvae and on the beneficial insects studied in these tests.

Investigations of the integration of chemical and biological control of a pest complex in any crop must include the effect of chemical treatment on the natural enemies of both major and minor pests. Chemicals of indiscriminate toxicity applied to suppress minor pests may, through the elimination of biological controls, permit the development of that pest into a major problem. Or, a major pest can become a ravaging menace where a chemical permits its survival while its biological controls are destroyed. Such unfavorable alterations in the treated area may require many months before the ecosystem returns to a favorable balance. In the case of the alfalfa weevil, *Hypera postica* (Gyll.), and the Egyptian alfalfa weevil, *H. brunneipennis* (Boheman), this consideration is of particular importance in California because the two weevil species are pests in early spring when many biological controls of field crop pests are coming out of diapause. Treatments of indiscriminate toxicity directed against these two minor pests can eliminate the weevils and also enemies of other pests.

The alfalfa weevil is found in a small area near the confluence of the Sacramento and San Joaquin rivers in the lowland of middle California and to the east and north of the Sierra Nevada Mountains (Michelbacher 1943, Hamlin *et al.* 1949). On the other hand, the Egyptian alfalfa weevil is found over most of southern California and appears to be gradually expanding its range of distribution (Reynolds *et al.* 1955).

Even though these two weevils are minor pests of alfalfa in California, they can be quite damaging to early alfalfa cuttings in certain localities. However, they are generally of minor importance compared with other insects (mainly aphids and lepidopterous larvae) that attack alfalfa in the same areas. Exceptions to this generalization are that the alfalfa weevil is often a major pest in the northeastern part of California and the Egyptian alfalfa weevil is one of the few insects attacking alfalfa along the southern coast. However, the total alfalfa acreage grown in these two areas is a very small per cent of the 1,200,000 acres grown annually in the State.

Because of climatic conditions, that is, mild winters and warm-to-hot, arid summers, the damaging activity period of both *H. postica* and *H. brunneipennis* occurs in late winter and during the spring. This is usually during the first and second cuttings, and chemical treatments are occasionally needed to prevent damage. The third, fourth, and fifth cuttings of the five annual cuttings in the central part of the State and the third through the eighth annual cuttings in the Imperial Valley are essentially weevil-free.

Both species are subject to a number of environmental factors that reduce the larval populations or eliminate the estivating adults. Consequently, chronic infestations are rare except in areas where parasite activity (*Bathyplectes curculionis* (Thoms.)) is restricted and the dia-

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[3] This study is a continuation of a research program of integrated control methods against pest insects. This particular phase of research was completed before the Pure Food and Drug Administration established a zero tolerance for heptachlor and heptachlor epoxide on alfalfa hay. Data regarding heptachlor and other compounds, and other data and conclusions, are presented that may be useful to researchers interested in developing the integrated control concept.

pausing quarters are undisturbed. The parasite *B. curculionis* is very important as a biological control agent on the alfalfa weevil in northern California and in other areas (Michelbacher 1943, Hamlin *et al.* 1949). This parasite is less important in the control of the Egyptian alfalfa weevil in southern California because of the partial immunity of the weevil to it. Immunity is effected through the lethal action of the weevil blood (phagocytosis) on the parasite eggs (van den Bosch & Dietrick 1959). Further, many adults of both species leave the alfalfa fields during late spring to diapause under the bark of nearby trees or in protected places along irrigation ditch banks. Some adults remain in the fields, but the frequent cutting of alfalfa during the hot summer apparently destroys many adults (Reynolds *et al.* 1955). Others are eliminated by the rapid rotation program whereby alfalfa in most of southern California is plowed under after 3 years' cropping and is plowed under after 5 years' cropping in most other areas. Likewise, the heavy disking, chemical weed spraying, and burning of weeds along irrigation ditch banks kill many of the diapausing adults that move to the edges of the fields. All these factors help to prevent chronic infestations. Because of these factors and because prophylactic treatments are generally not recommended for control of field crop pests in California, control measures are directed against economic larval populations rather than, as insurance or prophylactic treatments, against adults.

FIELD TESTS.—While continuing investigations of integrated control methods against pest insects, field tests were conducted to determine the effects of heptachlor and other materials on *B. curculionis*, a parasite of the alfalfa weevil and the Egyptian alfalfa weevil. Also evaluated was the effect of heptachlor and other materials on other parasites and predators which are not specifically enemies of the two weevil species, but are important enemies of other pests which attack alfalfa and other field crops. Heptachlor was used at the rate of 2.0 to 2.4 ounces per acre because the data of Reynolds *et al.* (1955) show that 1 to 2 ounces of heptachlor per acre will give adequate control of the Egyptian alfalfa weevil larvae, and the unpublished data of R. F. Smith (University of California, Berkeley) also show that the same dosage will control alfalfa weevil larvae.

In the field tests, all materials were applied as sprays; an airplane was used in the first test, and ground equipment was used for the two succeeding tests.

Test No. 1.—On March 11, 1959, sprays were applied by airplane to alfalfa in the Imperial Valley, California. Each treatment was replicated four times and each plot was 80 feet wide and 1,225 feet long. Five gallons of spray mixture were applied per acre on alfalfa that was approximately 20 inches tall.

The plots were sampled by using a vacuum suction machine, the original of which was constructed for collecting arthropods by Everett J. Dietrick and Evert I. Schlinger, University of California Citrus Experiment Station. Detailed descriptions of the vacuum suction machine and of the faunistic sampling method can be found in Dietrick *et al.* (1959, 1960). Figure 1 shows diagrammatic sketches of the vacuum suction machine and modified Berlese funnel. Figure 2 shows the duff and insects being placed in the modified Berlese funnel insect separators. Figures 1 and 2 appear with the permission of E. J. Dietrick. (See next page for Figs. 1 and 2.)

The major advantage of the vacuum suction machine method of sampling in comparison with a sweep net is that it permits the rapid collecting of a nearly complete quantitative measure of the insects from the ground surface to the top of the alfalfa plants. The collected arthropods may be easily separated by the modified Berlese funnels (fig. 2) and preserved in alcohol and counted at leisure. Since the samples are drawn from within a closed system, the sampling may be carried on under adverse weather conditions which is not possible with the insect sweep net because some insects crawl under the soil litter at night or in cold weather. For certain insect species, this sampling method is the only way to gather enough units to make a quantitative study (Dietrick *et al.* 1960). In test 1 five square feet were sampled per plot, or a total of 20 square feet per treatment. The summarized data are shown in table 1.

When the first sample was taken 24 hours after application, heptachlor applied at 2.0 ounces per acre had little or no effect on the convergent lady beetle, *Hippodamia convergens* G.-M., which is a major predator of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), and of the pea aphid, *Macrosiphum pisi* (Harris), in California. There

Table 1.—Effect of synthetic insecticides and *Bacillus thuringiensis* spore material on the Egyptian alfalfa weevil and a number of beneficial insects in alfalfa in the Imperial Valley, California.

	Days After Treatment and No. of Insects[b] per 20 Sq. Feet													
	Hippodamia convergens				*Orius tristicolor*				*Trioxys utilis*		*Chrysopa* spp.		*Hypera brunneipennis*	
	1		6		1		6		1	6	1	6	1	6
Material[a] and Ounces per Acre	A	L	A	L	A	N	A	N	A	A	L	L	L	L
Heptachlor, 2.0	2	23	3	47	3	17	4	41	25	64	0	2	5	0
Bacillus thuringiensis[c]	2	15	5	34	2	15	4	30	57	174	3	3	37	10
Parathion, 3.5	1	1	1	5	0	1	0	2	6	15	0	1	7	0
Phosdrin, 3.5	1	7	2	20	0	10	0	10	34	71	8	3	9	1
Untreated	3	18	3	31	1	7	6	25	48	210	4	2	42	10

[a] Sprays applied by airplane, March 11, 1959.
[b] A, adults; L, larvae; N, nymphs.
[c] Applied 14 ounces of *Bacillus thuringiensis* spore material per acre. Each gram contained 40 billion viable spores.

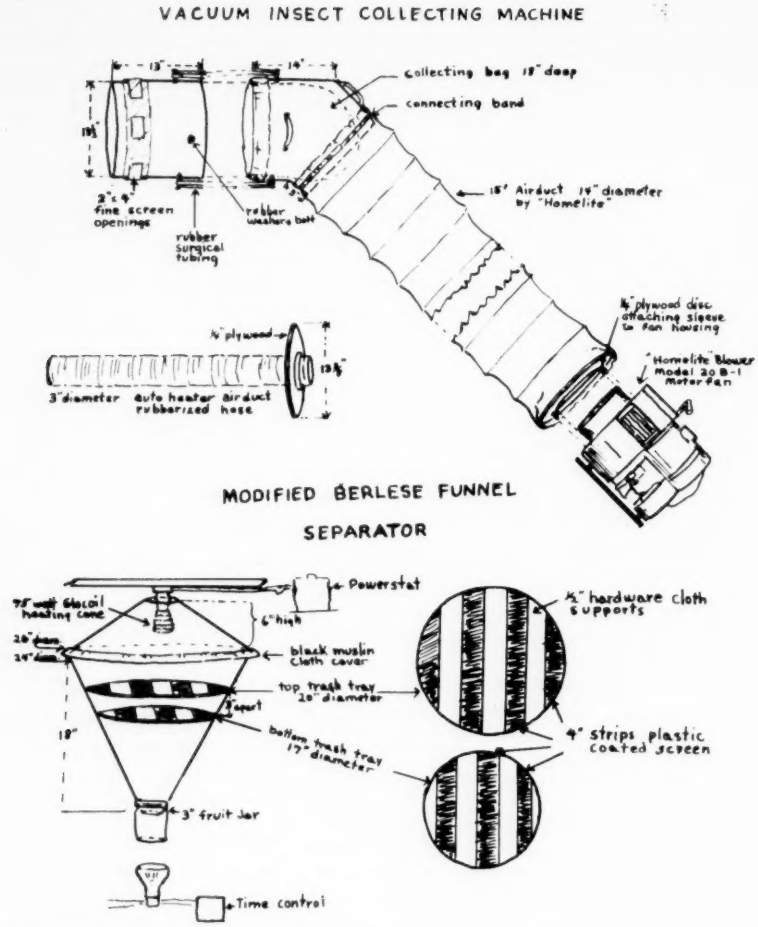
VACUUM INSECT COLLECTING MACHINE
collecting bag 19" deep
connecting band
15' Airduct 14" diameter by "Homelite"
2"x 4" fine screen openings
rubber surgical tubing
rubber washers both
¼" plywood disc attaching sleeve to fan housing
"Homelite" Blower model 20 B-1 Motor Fan
¼" plywood
3" diameter auto heater rubberized hose
MODIFIED BERLESE FUNNEL SEPARATOR
Powerstat
75 watt Glacoil heating cone
6" high
¼" hardware cloth supports
black muslin cloth cover
top trash tray 20" diameter
4" strips plastic coated screen
bottom trash tray 17" diameter
3" fruit Jar
Time control

FIG. 1.—Diagrammatic sketch showing the principal details of the vacuum insect-collecting machine and the modified Berlese funnel separators. (Sketch was drawn by and appears with the permission of E. J. Dietrick.)

FIG. 2.—Modified Berlese funnel separators.

Table 2.—Effect of parathion and heptachlor sprays on *Hypera brunneipennis*, the weevil parasite (*Bathyplectes curculionis*) and *Hippodamia convergens*, at Escondido, California.

Material[a] and Ounces per Acre	Days After Treatment and No. of Insects[b] per 80 Sweeps																			
	B. curculionis				*Hippodamia convergens*								*Hypera brunneipennis*							
	1	3	8	16	1		3		8		16		1		3		8		16	
	A	A	A	A	A	L	A	L	A	L	A	L	A	L	A	L	A	L	A	L
Parathion, 4.1	16	41	42	91	2	0	5	0	28	2	60	0	3	78	11	44	53	78	32	212
Heptachlor, 2.4	52	52	55	40	8	0	8	5	118	8	357	19	0	123	5	34	32	34	23	33
Untreated	47	55	87	80	15	2	5	5	105	8	298	46	4	286	12	229	45	476	90	611

[a] Sprays applied by ground equipment, March 24, 1959.
[b] A = adults, L = larvae.

also appeared to be no reduction in *Orius tristicolor* (White) (determined by P. D. Ashlock, U. S. National Museum) which is an important predator of thrips and spider mites. Heptachlor was moderately toxic to *Trioxys utilis* Mues., one of the important parasites of the spotted alfalfa aphid, and to *Chrysopa* spp., an important predator of a wide number of field crop pests. The data of Stern *et al.* (1960), showing that heptachlor applied at 2.4 ounces per acre by ground equipment had little, if any, effect on *H. convergens*, *O. tristicolor*, and *Chrysopa* spp., are essentially comparable to the results obtained in test 1.

Parathion applied at 3.5 ounces per acre was very toxic to *H. convergens*, *O. tristicolor*, *Chrysopa* spp., and *T. utilus*. Phosdrin® (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate 60% technical), also applied at 3.5 ounces per acre, was not nearly as toxic to these beneficial insects as parathion. The spore material of *Bacillus thuringiensis* Berliner had little, if any, effect on these beneficial insects.

One day after application, heptachlor and parathion gave, respectively, 84% and 60% reduction of the Egyptian alfalfa weevil larvae. Six days after treatment, no weevil larvae were collected in any of the plots treated with these two materials.

When the test was started most of the larvae were large, and 6 days after application there were approximately 75% fewer larvae in the untreated plots in comparison with the number collected 1 day after application. Unquestionably, many of the large larvae had pupated after the test began. Data obtained later (tests 2 and 3) and results reported by Reynolds *et al.* (1955) indicate that heptachlor and parathion were largely responsible for the decrease in larvae rather than a decrease because of pupation with heptachlor being more toxic to the larvae than parathion. Phosdrin was not as satisfactory as heptachlor or parathion in reducing the weevil population. However, 99% control of alfalfa weevil and the Egyptian alfalfa weevil larvae is not necessary under California conditions and may not even be desirable.

The spore material of *B. thuringiensis* applied at 14 ounces per acre (each gram contained approximately 40 billion viable spores) gave no reduction of the weevil larvae.

Test No. 2.—This test was conducted for a number of reasons. It was known that both heptachlor and parathion would give adequate control of the Egyptian alfalfa weevil. However, previous tests and the data of Stern *et al.* (1958) and of Stern & van den Bosch (1959) had shown that parathion at 2 to 4 ounces per acre was toxic to a wide variety of beneficial insects attacking field crop pests in California. On the other hand, heptachlor at 2.4 ounces per acre was relatively nontoxic to these beneficial insects (Stern *et al.* 1960). Further information was desired on the effect of these two compounds on the weevil parasite, *B. curculionis*. In addition, experimentation was needed to show that decreasing the population of weevil larvae while allowing the parasite *B. curculionis* to survive would subsequently increase the per cent parasitism of the weevil larvae surviving treatment. The reason for this is that a single egg oviposited in an Egyptian weevil larva tends to be phagocytised (van den Bosch & Dietrick 1959). Thus, decreasing the weevil population without a corresponding decrease of *B. curculionis* would increase the per cent parasitism of the weevil larvae because of superparasitism.

On March 24, 1959, sprays were applied by ground equipment to alfalfa at Escondido, California. Each treatment was replicated four times and each plot was 60 feet wide and 120 feet long. The alfalfa was about 24 inches tall and approximately 12 gallons of spray mixture were applied per acre. The plots were sampled by using a standard insect-sweeping net, each sweep describing a 180-degree arc in the alfalfa. The summarized data appear in table 2.

Heptachlor applied at 2.4 ounces per acre had little or no effect on the weevil parasite, *B. curculionis*, when the plots were sampled on the first and third days after application. On the 8th and 16th days after application there were fewer parasites in the heptachlor plots than in the untreated plots. However, in the plots treated with heptachlor most of the weevil larvae were killed and undoubtedly many of the parasites had migrated into the adjoining untreated areas where the host populations were undisturbed. Heptachlor also had little or no effect on *H. convergens*.

Parathion applied at 4.1 ounces per acre reduced the parasite population by approximately 66% one day after application. On the third and eighth days after application there were also fewer parasites in the parathion plots than in the untreated plots. Parathion also had a drastic effect on the convergent lady beetle. The direct effect of parathion on the convergent lady beetles was unques-

Table 3.—Effect of heptachlor and demeton sprays[a] on *Hypera brunneipennis*, the weevil parasite (*Bathyplectes curculionis*) and other parasites and predators at San Luis Rey, California.

Species and Stage		Treatment and Dosage per Acre and No. Insects per 20 Sq. Ft. on Day Indicated After Treatment								
		Heptachlor 2.3 ounces			Demeton 2.7 ounces			Untreated		
		1	3	5	1	3	5	1	3	5
Hypera brunneipennis	A[b]	11	9	4	38	18	36	35	44	42
	L	27	3	2	113	92	74	88	116	65
Bathyplectes curculionis	A	4	3	3	4	3	5	13	7	8
Hippodamia convergens	A	4	9	10	4	0	1	4	13	14
	L	25	25	34	19	15	10	25	41	43
Nabis ferus	A	0	0	0	1	0	1	1	0	0
	N	1	7	12	2	1	18	1	4	12
Trioxys utilis	A	3	3	1	5	2	1	6	11	3
Praon palitans	A	0	0	0	1	0	0	1	1	1
Aphelinus semiflavus	A	8	7	11	4	1	0	9	8	15
Chrysopa spp.	L	3	0	1	1	0	0	1	1	4
Orius tristicolor	A	0	1	2	2	3	9	1	2	4
	N	2	2	1	0	4	5	2	3	3

[a] Sprays applied by ground equipment, April 6, 1959.
[b] A, adults; L, larvae; N, nymphs.

tionably gone 16 days after application, and if the material had not eliminated the pea aphids, more adult beetles might have been collected on the last sampling day. Had parathion been sprayed on very large plots both the weevil parasite and the convergent lady beetles would have been drastically reduced over a wide area.

Heptachlor gave 86% reduction of the weevil larvae 8 days after treatment and 95% reduction 16 days after application. Control of the adults was not quite so effective although the data are masked by the fact that many adults were emerging. This fact is shown by the great increase of adult weevils in the untreated plots, and further by differences in color between newly emerged and diapausing females when the samples were counted. Control of the adults and of weevil larvae with parathion was not nearly so effective as with heptachlor.

On the 16th day after application the samples were brought into the laboratory to count and to dissect the weevil larvae for per cent parasitism. There was 72%, 69%, and 69% parasitism, respectively, in the untreated plots, and in the plots treated with heptachlor and with parathion. Thus, even though heptachlor had reduced the weevil larval population and had not decreased the weevil parasite population, the per cent parasitism of the surviving host population did not increase, despite the seemingly favorable relationship of the parasite over the host. Perhaps, sufficient time was not allotted for the parasite to play its intended role. Or, it may be that the plots were not nearly large enough for such an experiment, or perhaps, other unknown factors were present. Studies made with the intention of manipulating host-parasite populations are difficult experiments in entomology and more basic knowledge is needed to conduct them successfully.

Test No. 3.—On April 6, 1959, a second test was conducted to determine the effect of heptachlor on *B. curculionis*. The sprays were applied by ground equipment to alfalfa hay at San Luis Rey, California. Approximately 10 gallons of spray material were applied per acre, and each treatment was replicated four times. The plots were 60 feet wide and 110 feet long, and the alfalfa was approximately 14 inches tall. The plots were sampled by using the vacuum suction machine discussed in test 1. Five square feet were sampled per plot, or a total of 20 square feet per treatment. The data are summarized in table 3.

Heptachlor was applied at 2.3 ounces per acre and the subsequent reduction of *B. curculionis* varied between 58% and 68% during the sampling period. This was a greater reduction than in test 2 where there was little or no reduction of the weevil parasite.

Control of Egyptian alfalfa weevil larvae was satisfactory and thus the surviving parasites could continue their attack on the surviving weevil larvae or migrate to other fields and parasitize larvae there. Heptachlor also had little effect on *H. convergens*, *N. ferus*, *O. tristicolor*, and *Aphelinus semiflavus* Howard, a parasite of the spotted alfalfa aphid in the cooler and more humid areas of California. The effect of heptachlor on *T. utilus* was similar to the results obtained in test 1 where there was approximately 67% reduction of this important parasite of the spotted alfalfa aphid. *Chrysopa* spp. was reduced by about 75%.

The data of Stern & van den Bosch (1959) show that demeton applied at 2.5 ounces per acre is relatively nontoxic to many parasites and predators of field crop pests in California, and thus it is a preferred material for both

pea aphid and spotted alfalfa aphid control. It was used in this test to learn of its effect on *B. curculionis*. Demeton was applied at 2.7 ounces per acre and gave a moderate reduction of the weevil parasite. This is quite significant since pea aphid control with demeton usually occurs when the weevil parasite is most active. Thus, the weevil parasites surviving a demeton treatment for pea aphid control may continue attacking the weevil larvae.

One day after treatment, demeton appeared to have little effect on the convergent lady beetle. However, later samples showed a great decrease in lady beetles. Most likely, since the treatment eliminated the pea aphids, the convergent lady beetle adults then left the plots. Demeton appeared to have little effect on *N. ferus* and *O. tristicolor*, although it gave a moderate reduction of *T. utilus*. Its effect on *A. semiflavus* and *Chrysopa* spp. was drastic while it had no effect on the Egyptian alfalfa weevil.

The data obtained in these three experiments and that reported by Stern *et al.* (1960) show that heptachlor is a highly selective chemical when applied at dosages of 2 to 2.4 ounces per acre. These dosages will give satisfactory control of the alfalfa weevil and Egyptian alfalfa weevil larvae and permit the weevil parasite and many other parasites and predators of field crop pests to survive treatment. For these reasons it is a desirable material to control these two alfalfa pests and would serve a useful purpose in an integrated control program for alfalfa hay pests in California. Unfortunately or fortunately, as the case may be, the Pure Food and Drug Administration has recently established a zero tolerance of heptachlor and heptachlor epoxide on alfalfa hay. Nevertheless, since the compound is selective at low dosages and is thus unlikely to cause upsets or the resurgence of the treated species, heptachlor may eventually be utilized on alfalfa seed crops in certain areas and in other restricted situations.

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Factors Influencing Citrus Red Mite Populations on Navel Oranges and Scheduling of Acaricide Applications in Southern California[1]

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ABSTRACT

Trends of citrus red mite, *Panonychus citri* (McG.), populations on untreated trees, as well as the intervals during which potentially injurious populations were present on trees exposed to a variety of treatment schedules, were studied.

Seasonal citrus red mite population cycles occurred in the orchards in each of the 5 years. The specific time of each year in which these populations were increasing or decreasing varied from year to year depending on the seasonal growth cycles of the tree and the direct effects of weather extremes (hot, dry periods) on the mites.

Application schedules including two or three treatments were suggested. The number and timing of the applications were dependent on whether mite populations started to increase in late summer or fall following the annual early summer decline.

Studies which examine the factors influencing development or decline of citrus red mite, *Panonychus citri* (McG.), have recently been reviewed by Jeppson & Carman (1960). Jeppson and co-workers (1953, 1957a, 1957b) studied population trends of this mite in a Valencia orange orchard and in lemon orchards. They correlated population trends with seasonal meteorological changes and plant growth cycles and attributed certain population decreases to fairly short periods of combined high temperatures and low humidity. Field observations suggested that the population trends on navel oranges were sufficiently different from those on Valencia oranges and on lemons to warrant study.

MATERIALS AND METHODS.—The Washington navel orange orchards selected for this study were located in the Highgrove and upper Corona citrus districts of Riverside County. The Stevening orchard at Highgrove was planted in 1945 on rough lemon rootstock. The Jameson

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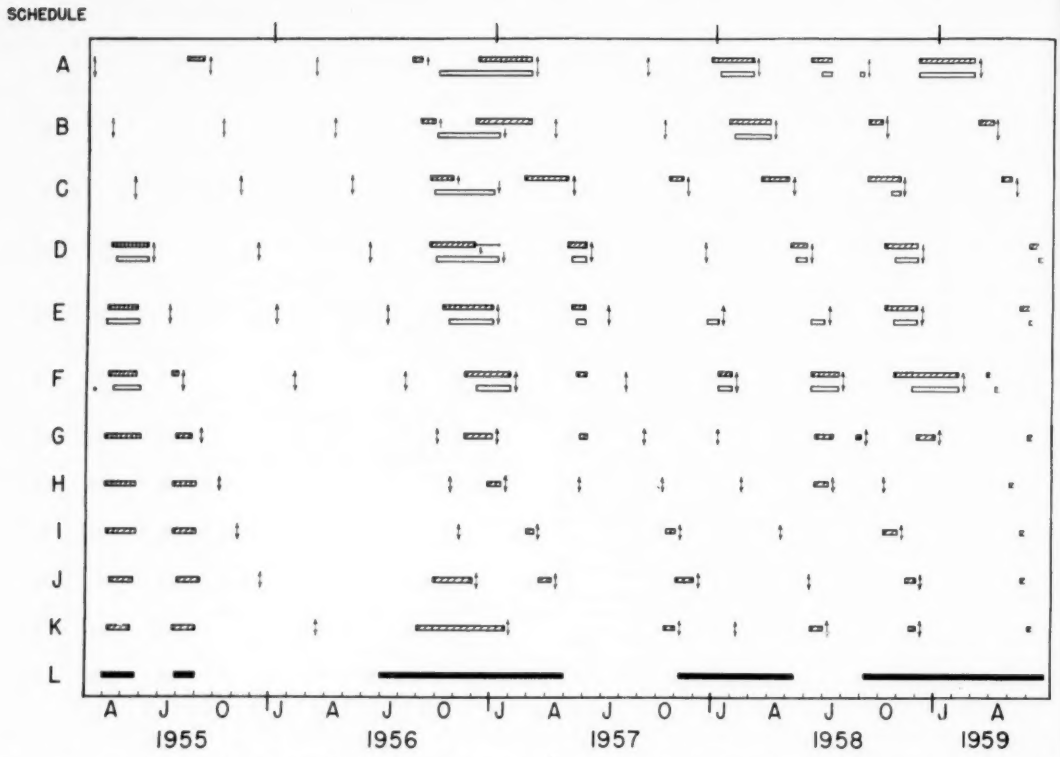

FIG. 1.—Intervals during which citrus red mite populations were sufficiently high in the Stevening orchard to produce injury. Plots were treated according to several schedules (see text). Shaded bar—treated with ovex 1955–56, Aramite 1957–59. Open bar—treated with demeton 1955–56, Kelthane 1957–59. Solid bar—untreated. ↕ = Scheduled application, schedules A–G, or as needed in schedules H–K. ↓ = Unscheduled treatment in A–G schedules of demeton, or Aramite. ↑ = Unscheduled ovex or demeton applications in A–G schedules. ——— = Interval from unscheduled treatment to time for scheduled application.

Company orchard located at Corona was planted in 1950 using sweet orange rootstock.

Treatments were applied to four replicates of 4-tree plots according to the following schedules:

Stevening Orchard

A. March and September	G. January and September
B. April and October	H. October and as needed
C. May and November	I. November and as needed
D. June and December	J. December and as needed
E. July and January	K. February and as needed
F. August and February	L. Check—untreated

Jameson Orchard

A. March and September	G. April and September
B. April and October	H. May and October
C. May and November	I. October and as needed
D. June and December	J. March and as needed
E. July and January	K. As needed
F. August and February	L. Check—untreated

The following toxicants were used. The number of ounces shown were added to 100 gallons of water. All formulations, except that of demeton which was an emulsifiable concentrate, were wettable powders.

Demeton	2	Tedion	4
Ovex	8	Malathion	10
Aramite	5	Parathion	6
Kelthane	4.5		

During 1955 one application of ovex was applied in the Stevening orchard (fig. 1, shaded bars) and two applications of demeton in schedules A to F (fig. 1, open bars). By October 1956 it became evident that two applications per year of each treatment were necessary to control mites in this orchard. At this time also, mite populations had become resistant to both ovex and demeton. Therefore, ovex was replaced by Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite) and demeton by Kelthane® (4,4′-dichloro *-alpha*-(trichloromethyl)-benzhydrol) and two applications of each of these acaricides were made per year from 1957 to 1959.

In the Jameson orchard two applications per year were scheduled for both treatments in schedules A to F (fig. 2). Ovex was used as the acaricide in one series of schedules from March 1955 to September 1956 (fig. 2, shaded bars). It was replaced by Aramite until November 1957 after which Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone) was used for the remainder of the experiment. Demeton was

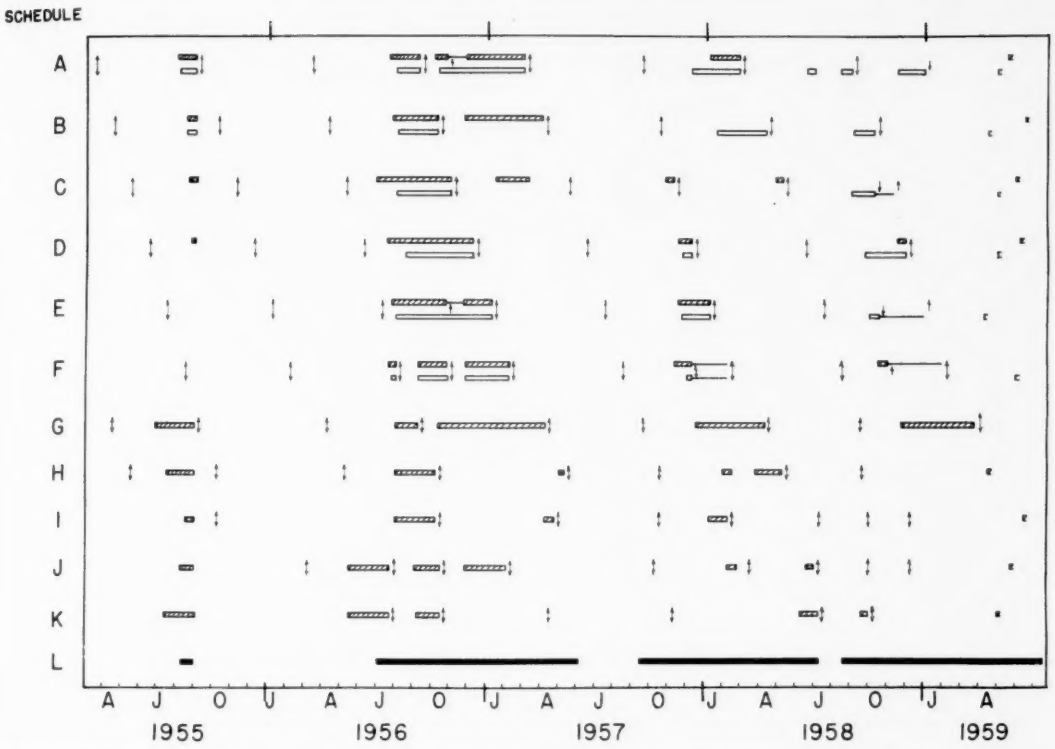

FIG. 2.—Intervals during which citrus red mite populations were sufficiently high in the Jameson orchard to produce injury. Plots were treated according to several schedules (see text). Shaded bar—treated with ovex 1955–56, Aramite 1956–57, or Tedion 1958–59. Open bar—treated with demeton 1955–56, Kelthane 1957–59. Solid bar—untreated. ↕ = Scheduled application, schedules A–H, or as needed in schedules I–K. ↓ = Unscheduled treatment in A–F schedules of demeton, or Aramite. ↑ = Unscheduled ovex, or Tedion application in A–F schedules. ——— = Interval from unscheduled treatment to time for scheduled application.

applied for mite control in the other series of schedules (open bars) from March 1955 to September 1956. By this time the mites in these plots had developed resistance to demeton; consequently, it was replaced by Kelthane.

In order to control California red scale, *Aonidiella aurantii* (Mask.), in the Stevening orchard, malathion was incorporated with ovex and later with Aramite sprays (fig. 1, shaded bars) applied May through October and parathion was added to May through October applications where either demeton or Kelthane was applied (open bars). Similarly, in the Jameson orchard malathion was included with ovex-Aramite-Tedion series in the May through October treatments (fig. 2, shaded bars) and parathion was added to May through October applications of demeton or Kelthane (open bars). DDT was applied to the Stevening orchard in August 1958 to control black scale, *Saissetia oleae* (Bern.).

A hygrothermograph located near the center of each orchard provided temperature and humidity records. A Livingston atmometer bulb was installed in each orchard to obtain comparative evaporation rates between the two orchards. They were placed beneath a standard hygrothermograph shelter located between two trees near the center of the orchard. A plywood board 9 inches square was placed 3 inches above the bulb to prevent exposure to the direct rays of the sun. Additionally, in the Stevening orchard an atmometer bulb was located near the trunk of one of the trees nearest the hygrothermograph shelter to ascertain the possible influence of transpiration on the rate of evaporation. Evaporation readings from all atmometers were taken each week at the time the hygrothermograph charts were changed.

The citrus red mite population on each tree was evaluated at weekly intervals by counting the number of adult mites on the four full-sized leaves nearest the tip of two sample twigs from each quadrant of the tree. Results are recorded as average number of mites on the four 32-leaf samples (Jeppson 1951).

RESULTS.—Citrus red mite population trends on untreated trees in the Stevening orchard from April 1955 to July 1959 are shown in figure 3. The number of hours in the week during which temperatures were above 95° F. are shown by the shaded bar. When the maximum temperature for the week reached 100° F. or above, the maximum is indicated below the bar. Atmometer records in millimeters per day are given for the weeks that this amount exceeded an average of 30 mm. daily. The amount of evaporation from the atmometer bulb placed under a

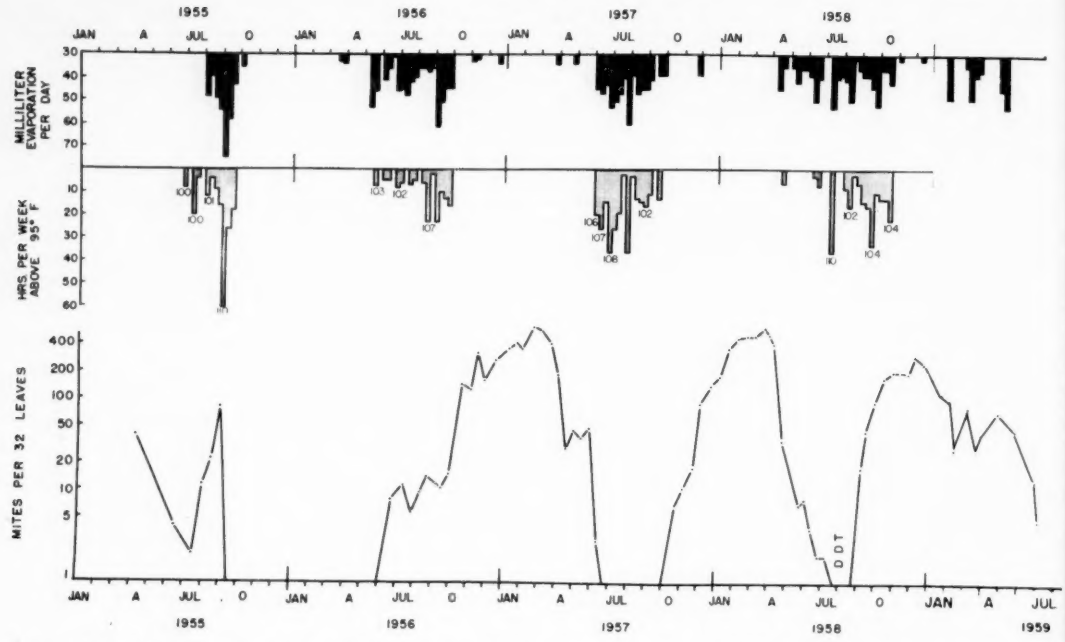

FIG. 3.—Records of the citrus red mite population on untreated trees in the Stevening orchard are shown in the line graph. The hours per week during which temperatures were above 95° F. are indicated in the shaded bar graph, with the maximum temperature indicated at the bottom of the bar. The solid bar graph shows the relative amount of evaporation in millimeters per day for weeks when this amount exceeded 30 mm. per day.

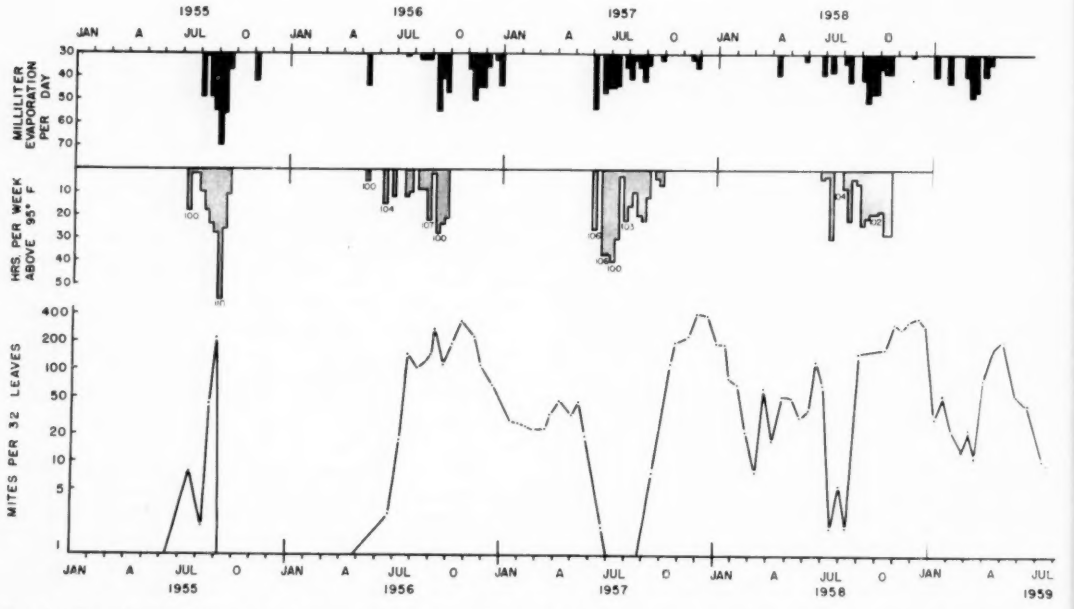

FIG. 4.—Records of the citrus red mite population on untreated trees in the Jameson orchard are shown in the line graph. The hours per week during which temperatures were above 95° F. are indicated in the shaded bar graph, with the maximum temperature indicated at the bottom of the bar. The solid bar graph shows the relative amount of evaporation in millimeters per day for weeks when this amount exceeded 30 mm. per day.

citrus tree averaged 63% as much as the one located in the shaded position between the trees.

The number of mites was considered abundant enough to produce damage when an average of 10 mites was found on a 32-leaf sample. Figure 1 (A–L) shows the intervals from April 1955 to July 1959 when mite populations were considered injurious in the various trial plots of the Stevening orchard.

Mite population trends on untreated trees as well as temperature and evaporation records in the Jameson orchard during the experimental period are shown in figure 4. The periods of damaging populations on trees treated as in the above schedules are plotted in figure 2 (A–L).

DISCUSSION.—Many factors may influence citrus red mite populations in California orchards. Some of these are climate, weather, natural enemies, host condition including leaf age, and mite feeding injury. The importance of each of these interrelated factors is difficult to evaluate. For example, in southern California navel producing areas, high temperatures are generally accompanied by relatively low humidities, and winds are usually associated with the more extreme low humidity conditions. When these latter conditions occur over a period of several days, not only have sharp declines in mite populations been observed, but the leaves and fruit have been made less favorable for mite development (Jeppson *et al.* 1957a). Under laboratory conditions Munger (1956) found that high temperatures had a more direct influence in arresting mite development than low humidities; however, changes brought about by such weather extremes on plant physiology which may indirectly have an effect on mite development have not been adequately studied. The authors, therefore, have chosen not to separate the effects of these weather factors on mite population trends in this discussion.

Although predaceous insects and mites are a major factor in the population dynamics in orchards in some districts of southern California (DeBach *et al.* 1951), the number of these beneficial species in the orchards under study was not a measurable factor in producing or altering the population trends recorded. Under present cultural conditions predaceous species are not sufficiently abundant to be a major factor influencing citrus red mite populations in most navel orange orchards in southern California.

Henderson & Holloway (1942) have shown that high populations of citrus red mite feeding on citrus leaves for an extended period of time produce conditions which are adverse to mite development. This has been observed to be a major cause in limiting the density of the mite populations and in population declines. The effect of this factor on population trends in the orchards studied will be discussed later in the paper.

The citrus red mite populations on untreated trees in both orchards under study increased in the summer or early fall and remained high until May or June of the following year, at which time a general population decline occurred. It is of interest to note that in 1955 a long period of high temperatures, including an extreme of 110° F., during the last of August and early September resulted in elimination of measurable mite populations in both orchards. Mites were not evident again until the following May or June. Shorter periods of high temperatures during June and July of 1957 and 1958 coincided with a sharp decline in mite infestations; however, a reinfestation occurred within 1 to 3 months.

Probably because of its location farther from the ocean, the Stevening orchard had longer intervals fairly free of mites than the Jameson orchard. With less influence from the sea breezes, there was a greater number of hours per day with high temperature and low humidity. These weather differences are most evident in the measurement of evaporation (compare solid bar graphs, figs. 3 and 4).

Although such extreme weather is unfavorable to citrus red mite populations, the correlation between the weather and decreased mite populations varied according to the time of year. During September and October a high evaporation rate, relative humidity below 20% and temperature up to 105° F., merely retarded development of populations. Similar weather conditions in May or June coincided with and probably resulted in the observed rapid decrease in mite population. It appears, therefore, that meteorological extremes may directly alter the annual citrus red mite population cycles but that other factors appear to be primarily responsible for the occurrence of these annual population cycles.

Evidence, both experimental (Henderson & Holloway 1942) and observable, indicates that the period of citrus-leaf growth between the very young and the mature leaves is most favorable to mite development. The major growth cycles of navel orange trees occur from March to May and again from September to November. These growth periods correspond with the periods of increasing or peak citrus red mite populations. It is likely then that seasonal growth cycles may be the major factors responsible for the general trends in citrus red mite populations. The amount of new growth and the time of year it occurs may vary from year to year according to weather conditions and such cultural practices as irrigation and pruning. These factors, together with the direct effects of weather on mites, could account for the observed variations in the population cycles.

By November the leaves are mature and therefore less favorable for mite development than when the leaves were young. At this time, however, the navel fruit provides favorable conditions for the development or maintenance of mite populations throughout the winter or until the fruit is harvested. Early harvesting may therefore materially reduce populations, thus removing a major source of infestation for the new growth which develops in the spring. Mites that reach these young leaves continue to increase until leaf maturity or adverse summer weather conditions, or the combination of their effects, causes populations to decline.

The declines in population occurring from November through April would appear to result from the "leaf conditioning" produced by a high mite density over a period of time; however, this apparently was not the major factor producing the population decline in May and June of 1957 and 1958 because simultaneously, mite infestations declined in treatment schedules F and G in the Stevening orchard in 1957 (See fig. 1, schedules F and G

for 1957) and in schedule A in 1958 in the Jameson orchard (See fig. 2, schedule A for 1958), where "population density" could not have been a major factor. High infestations over the extended period from October 1957 to July 1958 were followed by a sharp decline and by a rapid re-population in August. This indicates that the effects of "leaf conditioning" are soon lost in the absence of mites and certainly does not carry over to the next plant growth cycle.

Because of the time sequence of favorable growth conditions, citrus red mite populations on navel orange trees tend to be high throughout the fall, winter and early spring months. The variation in the time of year at which populations increase or decline and the extended period in which conditions are favorable for mite populations on navel oranges have not been conducive to development of calendar treatment schedules. The results of biannual treatments using different schedules (figs. 1 and 2) indicate that in some years one schedule was most effective; in other years a different schedule produced more effective results.

Figures 1 and 2 indicate graphically that treatments made on a calendar basis, during periods when populations were on the decline or when no mites were present, did not appreciably delay the onset of the next population cycle. As there was a decline in mite populations during May or June of each year, most acaricide treatments applied in March or April had to keep mite populations below the injurious level for only two months. Treatments applied at this time did not effectively delay the onset of the general population cycle which normally occurs during the summer or fall. Even when applications were made in July, August, or frequently even September, mite populations invariably reached injurious numbers during the fall and winter. Treatments made when first required in October and November, on the other hand, often kept mite infestations below injurious proportions until March or April when a short-term, spring treatment was often practical.

When summer conditions were unfavorable for mites, *i.e.*, with at least one long period having high temperatures and continuous low humidities, such as occurred during 1957, treatment could be delayed until October. Mite populations were effectively controlled by two applications per year, one in March or April, and the second one in October or November. However, during the years that had favorable summers, *i.e.*, with no more than a few days having high temperatures and low humidities, then injurious infestations appeared in July or August, as in 1956. Under these conditions, three treatments were necessary during the year: one in July or August, a second in October or November, and the third in March or April.

It is evident from the foregoing discussion that summer treatments cannot be timed by the calendar; therefore, periodic inspections appear to be advisable. When injurious populations appear between June and September any remedial treatment given should be followed by a late fall application to protect the orchards from citrus red mite injury.

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Control of Spider Mites and Black Spot on Roses with Acaricide-Fungicide Sprays and Dusts[1]

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ABSTRACT

Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite), demeton, and malathion were found to control the two-spotted spider mite (*Tetranychus telarius* L.) on roses. Maneb, zineb, ferbam, captan, sulfur-copper, and captan-sulfur were effective against black spot. Maneb and zineb were superior to ferbam and captan. Effective acaricide and fungicide treatments prevented damage to foliage and leaf drop, and resulted in the production of more flowers of better quality. Generally there were no significant differences between spray or dust methods of application. However, Aramite and malathion dusts did significantly reduce mite populations whereas sprays did not.

The control of spider mites and black spot of roses presents a difficult problem to the commercial grower and the home gardener. In many areas roses are attacked by these and other insect pests, such as aphids, Japanese beetle (*Popillia japonica* Newm.), and thrips, at the same time in the growing season. Combination acaricide-insecticide-fungicide sprays or dusts are most efficient for the control of these pests since the ingredients may be applied at one time.

A study to compare various acaricide-fungicide combinations was begun at Beltsville, Md., in 1950. Control has been obtained with dusts containing the acaricides Aramite (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite), malathion, or ovex and the fungicides maneb, ferbam-sulfur, sulfur-copper, captan, or zineb (Smith *et al.* 1953, McClellan *et al.* 1955). Demeton in spray combinations with maneb or zineb has also been found promising. When plant damage and loss of foliage owing to spider mites or black spot were prevented, there was a significant increase in flower production. Control of black spot was shown to affect early flower production the following year (McClellan *et al.* 1957).

The poor performance of Aramite and malathion in dusts containing captan led to laboratory investigations which revealed that Attaclay, a common diluent for captan, reduced the activity of the acaricide (Smith *et al.* 1955). In 1956 an experiment was conducted to evaluate combination sprays or dusts for the control of the two-spotted spider mite (*Tetranychus telarius* L.) and the black spot fungus (*Diplocarpon rosae* Wolf).

METHODS.—The experiment was conducted in randomized blocks consisting of 36 treatments replicated four times. Each plot contained six plants, three each of the varieties Helen Traubel and Red Radiance. The plants were set 2½ feet apart with 5 feet between plots, and mulched with 1 to 2 inches of sawdust. The fertilizers ammonium nitrate and 5-10-5 were applied as needed and the roses maintained in good growing condition by periodic irrigations throughout the season.

Thirty of the treatments were in a factorial arrangement of sprays and dusts consisting of no acaricide, Aramite, or malathion combined with no fungicide, ferbam, zineb, maneb, or captan. The others were Aramite-sulfur-copper (oxysulfate), malathion-captan-sulfur, and captan-sulfur dusts, and demeton sprays with or without ferbam or maneb.

In the sprays each acaricide (except demeton) and fungicide was used at the rate of 2 pounds of wettable powder in 100 gallons. The percentages of active ingredients in these powders were: Aramite 15, malathion 25, ferbam 76, zineb 65, maneb 70, captan 50. The demeton was in a 26.2% emulsifiable concentrate used at 1.5 pints in 100 gallons. All sprays also contained 1 pound of DDT, 0.5 pound of lindane, and 0.5 pint of Santomerse, an additional wetting agent, per 100 gallons.

In the dusts the percentages of active ingredients were: Aramite 1.5, malathion 4, ferbam 7.6, zineb 6.5, maneb 7, captan 10, copper 3.4 and sulfur 25. All dusts contained 5% of DDT and 1% of lindane. The diluent was pyrophyllite. Sprays were applied with 2-gallon compressed-air sprayers, and dusts with plunger-type hand dusters. The treatments were made weekly from the end of June to September 15, except that demeton sprays were applied every other week.

Red Radiance roses were infested with mites in May, several weeks before treatments were begun, to establish a uniform infestation. Helen Traubel roses were sprayed with spore suspensions of black spot fungus twice in July.

The flowers of both varieties were cut just above the first five-leaflet leaf twice a week in July, August, and September, and records were kept of their weight and number. Mite counts were made on July 13, July 31, and September 11. Five leaf discs, $\frac{9}{16}$-inch in diameter, were taken at random from the Red Radiance variety in each plot. Living mites of all stages were recorded. The Helen Traubel variety was rated on the basis of the percentage of foliage showing black spot lesions on August 9 and September 14.

RESULTS.—*Acaricides.*—The effects of the treatments on mites infesting Red Radiance roses are shown in table 1. In June and July Aramite, malathion, or Aramite-copper-sulfur dusts, demeton sprays alone or in combination with ferbam or maneb had fewer mites than the untreated check. In September Aramite and malathion dusts and demeton-ferbam sprays reduced mite populations whereas the demeton and demeton-maneb sprays and Aramite-copper-sulfur dusts did not. Throughout the season Aramite or malathion dusts and demeton sprays alone or in combination with ferbam had fewer mites than the untreated checks.

In September the yields from the Aramite and malathion spray plots and the Aramite dust plots were higher than those from the untreated checks. For the entire season those from the Aramite, malathion, demeton-ferbam, and demeton-maneb spray plots and the Aramite dust plots were higher than from the untreated check plots (table 2).

[1] Accepted for publication July 12, 1960.

Table 1.—Numbers of two-spotted spider mites per Red Radiance rose plot receiving various spray and dust treatments.

Treatment	Sprays			Dusts		
	July 13	July 31	September 11	July 13	July 31	September 11
Check	31	194	290	80	116	180
Ferbam	48	67	88	99	146	122
Zineb	114	133	125	188	121	147
Maneb	83	84	131	155	187	236
Captan	83	146	189	153	189	416
Mean	72	125	165	135	152	220
Aramite—						
Alone	27	119	244	13	23	103
Plus ferbam	16	62	99	51	71	144
Plus zineb	100	9	103	22	182	156
Plus maneb	68	94	127	21	21	85
Plus captan	26	101	154	28	26	62
Mean	47	77	145	19	65	110
Malathion—						
Alone	110	177	203	78	92	240
Plus ferbam	75	101	117	21	86	63
Plus zineb	201	45	224	59	59	83
Plus maneb	69	39	81	66	147	137
Plus captan	88	94	140	63	58	151
Mean	109	91	153	57	88	135
Demeton—						
Alone	0	2	98	—	—	—
Plus ferbam	2	11	38	—	—	—
Plus maneb	24	5	150	—	—	—
Aramite-sulfur-copper	—	—	—	11	47	95
Malathion-captan-sulfur	—	—	—	113	80	133
Captan-sulfur	—	—	—	67	202	109
L.S.D. at 5% level:						
To compare means	42	49	73	42	49	73
To compare any treatment combination	95	110	164	95	110	164
To compare treatment combinations vs. means	73	78	127	73	78	127

Table 2.—Yield of Red Radiance rose flowers following various acaricide-fungicide sprays and dusts.

Treatment	Sprays		Dusts	
	Grams	Number	Grams	Number
Check	819	137	720	124
Ferbam	800	137	897	153
Zineb	829	148	939	151
Maneb	735	108	1,126	183
Captan	952	160	959	147
Mean	827	138	928	152
Aramite—				
Alone	876	134	1,460	219
Plus ferbam	1,198	171	1,087	160
Plus zineb	904	131	1,328	185
Plus maneb	1,398	207	1,278	189
Plus captan	1,140	168	1,046	145
Mean	1,103	162	1,240	180
Malathion—				
Alone	982	158	1,032	167
Plus ferbam	1,032	154	1,062	158
Plus zineb	1,350	210	867	135
Plus maneb	966	156	1,182	179
Plus captan	964	145	989	157
Mean	1,059	165	1,026	159
Demeton—				
Alone	1,058	156		
Plus ferbam	1,383	202		
Plus maneb	1,656	217		
Aramite-copper-sulfur	—	—	1,060	163
Malathion-captan-sulfur	—	—	1,031	164
Captan-sulfur	—	—	918	142
L.S.D. at 5% level:				
To compare mean differences	196	27	196	27
To compare any treatment combination differences	438	60	438	60

Fungicides.—All fungicides tested gave good control of black spot (table 3). Maneb and zineb were outstanding, although ferbam and captan were also effective. Captan and captan-sulfur dusts reduced infections but were inferior to maneb and zineb sprays and dusts.

No significant differences in the yields of Helen Traubel roses owing to fungicide treatments were evident until September, when plots receiving zineb sprays and dusts and maneb dusts showed significantly higher yields than the untreated check. The total yields for the season were also higher in plots receiving maneb or zineb sprays or dusts (table 4).

Sprays vs. Dusts.—Throughout the season spider mite control was most efficient with dusts of Aramite or malathion (table 1). However, no significant differences between dusts and sprays were evident (table 5) except for malathion on July 13 when the dust was more effective (table 6).

Black spot was controlled with either sprays or dusts of effective fungicides alone or in combination with acaricides. No difference in yield of Helen Traubel roses was evident which could be attributed to either method (table 5).

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Table 3.—Black spot severity in Helen Traubel rose plants as affected by various fungicide treatments.[a]

Treatment	August 9		September 14	
	Spray	Dust	Spray	Dust
Check	76	77	78	78
Aramite	79	72	85	80
Malathion	76	75	88	88
Mean	77	75	84	82
Ferbam—				
Alone	36	33	40	18
Plus Aramite	21	29	23	33
Plus malathion	21	20	23	18
Mean	26	27	29	23
Zineb—				
Alone	14	27	15	25
Plus Aramite	11	18	10	15
Plus malathion	17	23	18	23
Mean	14	23	14	21
Maneb—				
Alone	23	20	20	25
Plus Aramite	12	14	15	10
Plus malathion	18	16	8	10
Mean	18	17	14	15
Captan—				
Alone	33	41	20	33
Plus Aramite	31	21	25	20
Plus malathion	35	22	25	35
Mean	33	28	23	29
Demeton—				
Alone	77		78	—
Plus ferbam	28		20	—
Plus maneb	12		18	—
Copper-sulfur-Aramite	—	20	—	13
Captan-sulfur	—	25	—	43
Captan-sulfur-malathion	—	31	—	38
L.S.D. at 5% level:				
To compare mean differences	5		7	
To compare any treatment combination differences	12		17	
To compare any treatment combination vs. mean differences	9		13	

[a] Maximum severity = 100, none = 0

Table 4.—Yield of Helen Traubel rose flowers following various acaricide-fungicide sprays and dusts.

TREATMENT	SPRAY		DUST	
	Grams	Number	Grams	Number
Check	1,086	140	838	114
Aramite	807	113	851	111
Malathion	943	124	1,061	146
Mean	945	126	917	124
Ferbam—				
Alone	775	108	1,132	124
Plus Aramite	1,228	143	1,087	123
Plus malathion	1,164	142	1,034	132
Mean	1,056	131	1,084	126
Zineb—				
Alone	1,269	141	1,190	144
Plus Aramite	1,195	146	1,239	149
Plus malathion	1,281	148	1,082	133
Mean	1,248	145	1,170	142
Maneb—				
Alone	1,088	134	1,292	157
Plus Aramite	1,138	132	1,279	146
Plus malathion	1,375	169	1,234	137
Mean	1,200	145	1,268	147
Captan—				
Alone	1,106	140	1,134	138
Plus Aramite	992	126	1,178	147
Plus malathion	1,164	141	1,139	152
Mean	1,087	136	1,150	146
Demeton—				
Alone	927	119		
Plus ferbam	1,068	125		
Plus maneb	1,209	140		
Sulfur-copper-Aramite	—	—	983	128
Captan-sulfur	—	—	1,316	167
Captan-sulfur-malathion	—	—	1,213	142
L.S.D. at 5% level:				
To compare mean differences	245	N.S.	245	N.S.

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Table 5.—Spray vs. dust methods affecting mite populations, black spot infections, and yield per plot of Helen Traubel and Red Radiance roses.

METHOD OF APPLICATION	MITE POPULATION			BLACK SPOT RATING		NUMBER OF FLOWERS	
	July 13	July 31	September 11	August 9	September 14	Red Radiance	Helen Traubel
Spray	76	154	98	28	33	82	92
Dust	71	155	101	28	34	90	91

Table 6.—Numbers of mites on July 13 in rose plots treated with sprays or dusts.[a]

METHOD OF APPLICATION	NO ACARICIDE	ARAMITE	MALATHION
Spray	72	47	109
Dust	135	19	58
L.S.D. at 5% level	—	—	42

[a] Mean for 60 observations.

Hercules AC-5727 as a Residual Spray for Adult Mosquitoes[1]

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ABSTRACT

Residues of Hercules AC-5727 (*m*-isopropylphenyl methylcarbamate) were highly effective against adults of *Anopheles quadrimaculatus* Say, *A. albimanus* Wied., *Aedes taeniorhynchus* Wied., and *A. aegypti* (L.). In laboratory tests with *A. quadrimaculatus* on plywood panels, dosages as low as 1 mg. per square foot produced 100% mortality for at least 20 weeks. The compound was better than DDT, malathion, dieldrin, or Bayer 29493 (Baytex®; *O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate). Its outstanding characteristic is the rapidity with which it acts, for a 1-minute exposure to deposits of 100 mg. per square foot consistently produced 100% knockdown within 5 to 10 minutes throughout the first month. Hercules AC-5727, malathion, and DDT did not have long residual toxicity on whitewash or sun-dried brick.

In buildings naturally infested with *A. quadrimaculatus*, Hercules AC-5727 and DDT were still giving excellent protection after 18 weeks. Both were more durable than malathion.

Reports of resistance to DDT and/or dieldrin in *Anopheles* mosquitoes have been appearing with increasing frequency. Some of these reports were included by Brown (1958) in his Monograph on Insecticide Resistance in Arthropods. The problem is not merely local, but is becoming prevalent throughout the world in areas where residual spray programs have been conducted for several years.

In an effort to find other materials that are more effective than the chlorinated hydrocarbons, an extensive program of testing is being conducted at the Orlando, Florida, laboratory of the Entomology Research Division. Recently LaBrecque *et al.* (1960) reported that in preliminary tests Hercules AC-5727 (*m*-isopropylphenyl methylcarbamate) appeared very promising for use as a residual insecticide against *Anopheles quadrimaculatus* Say. This compound is a gray crystalline material with a slight odor and a melting point of 76° C. It is soluble in acetone, alcohol, and xylene but insoluble in water. It is not yet available commercially, but is being prepared in small quantities for testing purposes. The acute oral LD-50 to white rats was first reported to be about 45 mg. per kg., but more recently as about 15 mg./kg.

This paper reports the results of additional studies with this and other materials against adult mosquitoes. Most of the tests were run under laboratory conditions, but practical treatments were also made in infested buildings. In the laboratory evaluations, acetone solutions of the compounds were sprayed on various surfaces at several

[1] Accepted for publication July 14, 1960.

Table 1.—Toxicity to *Anopheles quadrimaculatus* of various dosages of Hercules AC-5727 applied to plywood panels.

Dosage (mg./sq. ft.)	Exposure (minutes)	Per Cent Kill in 24 Hours After Aging for—							
		1 Day	1 Week	2 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks
100	1	100	100	100	100	100	67	20	0
	5	—	—	—	—	—	100	97	71
	15	—	—	—	—	—	—	100	100
50	1	100	100	65	15	14	14	17	4
	5	—	—	100	65	92	30	55	6
	15	—	—	—	100	100	84	100	100
	30	—	—	—	—	—	93	100	—
	60	—	—	—	—	—	100	—	—
25	1	88	17	29	9	4	—	15	3
	5	100	100	97	73	24	10	41	4
	15	100	—	100	100	98	11	100	37
	30	—	—	—	—	100	100	100	100
10	1	90	38	5	2	0	—	13	—
	5	100	100	67	71	29	7	17	3
	15	100	—	100	100	98	14	80	22
	30	—	—	—	—	100	90	100	93
	60	—	—	—	—	—	100	—	75
	120	—	—	—	—	—	—	—	100
1	5	38	14	5	—	11	—	3	11
	15	100	95	26	33	53	18	42	19
	30	100	94	79	94	97	82	100	40
	60	—	100	100	61	100	100	—	95
	120	—	—	—	100	—	—	—	100

dosages. At intervals after treatment approximately 50 mosquitoes (mixed sexes) were exposed on the treated surfaces under half sections of petri dishes for periods ranging from 1 to 120 minutes. The full range of exposures was not used each time, but an effort was made to achieve a range of mortalities that reached or approached 100%. After exposure the insects were transferred to untreated screen holding cages, furnished cotton that had been saturated with 10% honey solution, and held for 24-hour mortality counts.

The strain of *A. quadrimaculatus* used in the laboratory tests has developed a high level of resistance to dieldrin in the course of many years of laboratory maintenance. At the time these tests were made the larvae showed about 100-fold resistance, and adult mortality did not exceed 16% following a 1-hour exposure on papers treated with 0.8% dieldrin, using the World Health Organization standard test kit.

Effectiveness of Different Dosages—Tests were made to determine the effectiveness of Hercules AC-5727 at 1 to 100 mg. per square foot on plywood against *A. quadrimaculatus*. The results appear in table 1. Hercules AC-5727 was highly effective at all dosages. Treatments as low as 1 to 10 mg. per square foot still were causing 75% to 100% mortality in 24 hours the 20th week after application at exposures of 60 to 120 minutes. Increasing the dosage reduced the exposure time required to kill; dosages of 25 to 100 mg. were still producing 100% mortality with exposures of 15 and 30 minutes during the 20th week. A dosage of 100 mg. per square foot caused 97% to 100% mortality after 1-minute exposures for 8 weeks and after 5-minute exposures for 16 weeks. As a fresh application, the 50-mg. deposit produced 100% knockdown before the insects could be removed from the treated surface during a 5-minute exposure and within 30 minutes after a 1-minute exposure. Exposures of 5 minutes' duration on 25- and 10-mg. residues were sufficient to cause total knockdown within 30 minutes. The time required for knockdown gradually increased as the treatments aged and lost some of their effectiveness.

Species Susceptibility.—To determine if other species of mosquitoes were as susceptible as *A. quadrimaculatus*, this compound was also tested against adults from laboratory colonies of *Aedes taeniorhynchus* Wied., *A. aegypti* (L.), and two strains of *Anopheles albimanus* Wied.[2] The Salvador Selected strain was collected in January 1959 in buildings in Santo Domingo, El Salvador, where dieldrin no longer gave good control. Since that time this strain has been reared under laboratory conditions without further exposure to insecticides. The Mexico strain has been reared in the laboratory for about 10 years without exposure to insecticides. Adults of these four species were exposed for 1 and 5 minutes to plywood panels that had been treated 3 to 10 days previously with 100 mg. of Hercules AC-5727 per square foot. The *Anopheles* species were also tested on 10- and 1-mg. deposits of the insecticide.

All four species were highly susceptible to this insecticide. The 5-minute exposure on the 100-mg. treatments affected the mosquitoes so rapidly that none were able to fly when removed from the boards. A 1-minute exposure on the same surfaces was somewhat slower in action, but produced complete knockdown within 30 minutes. It was not possible to distinguish between the susceptibility of

[2] The *Anopheles albimanus* adults were provided by the Pan American Sanitary Bureau through L. E. Rozeboom, and Jesse H. Hobbs from colonies maintained at Johns Hopkins University.

Table 2.—Relative toxicity to *Anopheles quadrimaculatus* of various insecticides on plywood panels.

Insecticide and Dosage (mg./sq. ft.)	Exposure (minutes)	Per Cent Kill in 24 Hours After Aging for—							
		1 Day	1 Week	2 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks	20 Weeks
DDT									
100	5	21	9	73	29	50	29	50	3
	15	59	60	100	57	96	78	89	36
	30	100	100	100	100	100	100	100	85
	60	—	—	—	—	—	—	—	100
10	15	29	15	27	23	53	6	0	—
	30	37	23	86	94	89	33	25	3
	60	100	88	100	98	100	97	100	32
	120	—	92	—	100	—	100	100	100
Malathion									
100	5	24	44	13	25	31	7	5	17
	15	97	100	78	74	61	31	79	97
	30	100	100	97	100	100	100	100	100
	60	—	—	100	—	—	—	—	—
10	5	2	13	—	—	—	—	—	—
	15	88	54	0	7	49	9	7	7
	30	100	100	74	68	95	97	71	16
	60	—	—	95	100	100	100	100	92
	120	—	—	100	—	—	—	—	100
Dieldrin									
100	5	57	40	45	48	—	29	18	4
	15	24	57	75	74	29	39	54	9
	30	77	83	81	92	96	100	52	38
	60	100	87	100	90	100	100	100	96
	120	—	98	—	98	—	—	—	100
10	5	21	32	30	—	—	—	—	—
	15	62	48	47	25	14	16	30	—
	30	30	50	69	95	89	67	35	16
	60	53	64	67	59	100	100	82	46
	120	76	100	100	86	100	—	100	100
Bayer 29493									
100	5	100	100	100	100	100	50	50	4
	15	100	100	100	100	100	97	84	8
	30	—	—	—	—	—	100	100	65
	60	—	—	—	—	—	—	—	96
	120	—	—	—	—	—	—	—	100
10	5	45	—	11	7	—	—	—	—
	15	100	12	46	13	11	—	—	—
	30	100	100	52	15	33	30	10	9
	60	—	100	27	50	98	100	100	53
	120	—	—	91	100	100	—	—	100
Hercules AC-5727									
100	1	100	100	100	100	100	67	20	0
	5	—	—	—	—	—	100	97	71
	15	—	—	—	—	—	—	100	100
10	1	90	38	5	2	0	—	13	—
	5	100	100	67	71	29	7	17	3
	15	100	—	100	100	98	14	80	22
	30	—	—	—	—	100	90	100	93
	60	—	—	—	—	—	100	—	75
	120	—	—	—	—	—	—	—	100

the various species. Both species of *Anopheles* also appeared to be equally susceptible to the 10-mg. deposit, for the 30-minute knockdown was always 100% following the 5-minute exposure and ranged between 71% and 79% for the three strains after the 1-minute exposure. Some evidence was obtained in the tests with the 1-mg. dosage that *A. quadrimaculatus* was a little less susceptible than *A. albimanus*. A 5-minute exposure on this treatment caused 30-minute knockdown of 97% to 100% with the two strains of *A. albimanus*, but only 14% with *A. quadrimaculatus*. In every test the 24-hour mortality was 100%.

COMPARISONS WITH OTHER INSECTICIDES.—*A. quadrimaculatus* was exposed on plywood panels to compare Hercules AC-5727 with DDT, malathion, dieldrin, and Bayer 29493 (Baytex®; *O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate), which are now being used or being considered for use in large-scale malaria control campaigns. Because of the high effectiveness of Hercules AC-5727 at low dosages, all insecticides were applied at both 10- and 100-mg. per square foot. The results are presented in table 2. Hercules AC-5727 was the most effective. Its superiority was most apparent at 10 mg. per square foot, exposures of 5 or 15 minutes consistently producing 98% to 100% mortality for 8 weeks and exposures of 30 minutes causing at least 90% mortality after 20 weeks. The 10-mg. deposit of Bayer 29493 appeared to

be better than similar deposits of the other three compounds as a fresh application but not after 2 weeks. The 100-mg. deposits of both Hercules AC-5727 and Bayer 29493 caused total mortality within 24 hours throughout the first 8 weeks of aging at the shortest exposure used. However, by the 12th week it became apparent that Hercules AC-5727 was the more durable of the two. There was no great difference between DDT and malathion at 100 mg. throughout the 20-week period and these compounds were only slightly more effective than dieldrin.

Hercules AC-5727 was more rapid in its action than the other compounds. The highest knockdown in 30 minutes with DDT, malathion, dieldrin, or Bayer 29493 was 21%, even though the mosquitoes were exposed continuously on the treated surfaces. In contrast, a 1-minute exposure on the 100-mg. deposit of Hercules AC-5727 consistently produced 100% knockdown within 5 to 10 minutes throughout the first month. In most cases the actual period of contact was much less than 1 minute, as the mosquitoes usually flew continuously and only touched the insecticide momentarily. When the exposures were increased to 5 minutes, the insects did rest quietly on the treated surfaces during part of the exposure period. Although DDT residues have been highly effective as a malaria control measure, they have been so slow in action that people in treated houses often complained because annoyance was not alleviated. Hercules AC-5727 appears to act sufficiently rapidly to eliminate most of the annoyance.

EFFECTIVENESS ON CLAY AND WHITEWASH.—Malathion has been reported to lose effectiveness rapidly on alkaline surfaces, particularly whitewash. Difficulty has also been experienced with DDT on various types of mud walls. A series of tests was made to compare the durability of Hercules AC-5727, malathion, and DDT on sun-dried red mud bricks (pH 4.77), and on baked red clay (flowerpots, pH 7.91) either unpainted or painted with whitewash (pH 12.25) 1 week before spraying. All treatments were applied at 100 mg. per square foot, and the exposure period was maintained at 1 hour throughout most of the test. When a 1-hour exposure failed to produce high mortality during the fourth and eighth weeks, the exposures to Hercules AC-5727 and malathion on the sun-dried brick were increased to 2 hours. Results of these tests are shown in table 3. All three compounds remained highly effective on the unpainted baked clay throughout the 12-week test period. However, none had long residual toxicity on whitewash or the sun-dried brick. Malathion was almost completely ineffective the day after the sprays were applied. Hercules AC-5727 was highly toxic the day after treatment, but was worthless by the fourth week. Although DDT was somewhat better than these two materials, it was causing less than 50% mortality by the eighth week.

Table 3.—Mortality of *Anopheles quadrimaculatus* exposed for 1 hour on various surfaces treated with 100 mg. of insecticide per square foot.

Surface	Insecticide	Per Cent Kill in 24 Hours After Indicated Weeks of Aging—			
		0	4	8	12
Whitewashed baked clay	Hercules AC-5727	100	0	0	—
	DDT	100	88	33	10
	Malathion	8	—	—	—
Unwhitewashed baked clay	Hercules AC-5727	100	100	100	100
	DDT	100	100	100	94
	Malathion	100	100	100	92
Sun-dried mud brick	Hercules AC-5727	100	12[a]	2[a]	—
	DDT	100	19	34	—
	Malathion	13	14[a]	8[a]	—

[a] Exposure period increased to 2 hours.

FUMIGATION ACTION.—To ascertain whether Hercules AC-5727 acted as a fumigant, 2 grams of the chemical were placed in a pile on a clean sheet of paper, inside a metal ring 2 inches in diameter and 1 inch high, and covered with galvanized screen wire. The chemical was at least one-half inch below the screen. A cage containing 75 *A. quadrimaculatus* adults was placed on top of the screen wire and a 1-gallon glass jar inverted over it. The knockdown was 0% after 30 minutes, 3% after 60 minutes, 95% after 120 minutes, and 100% after 150 minutes. Another cage of mosquitoes was treated similarly, but no insecticide was placed inside the metal rim. There was no mortality after 24 hours in this untreated control.

The rate of knockdown was so much slower in this test than in the direct-contact tests that fumigating action does not appear to be primarily responsible for the mortality attained with Hercules AC-5727 in the previously discussed studies.

DURABILITY IN BUILDINGS.—Because of the very encouraging results obtained under laboratory conditions, Hercules AC-5727 was compared with malathion and DDT as residual treatments applied to the walls and ceilings of small buildings naturally infested with adults of *Anopheles quadrimaculatus*. All buildings were wooden structures located in the vicinity of a lake near Bushnell, Florida. Hercules AC-5727 was used at the rate of 100 mg. per square foot in a small barn that housed a calf and a flock of chickens, and at 50 mg. per square foot in a small pump house. The malathion and DDT were applied in sheds on premises where chickens, pigs, and cows were being maintained. The buildings were treated on February 10, 1960, with emulsions containing 1% or 0.5% of toxicant. The emulsions of Hercules AC-5727 and DDT were prepared from concentrates containing 25% of insecticide, 65% of xylene, and 10% of Triton X-100, and the malathion from a commercial emulsion concentrate. Two months after treatment the animals were removed from the premises on which the DDT-treated building was located.

Pre- and posttreatment counts of the mosquitoes resting in these buildings were made once or twice each week in the middle of the day. The pretreatment counts were started at least 1 month prior to spraying. Four untreated buildings served as checks. The number of mosquitoes in the untreated buildings averaged 169 in January, 62 in February, 45 in March, 148 in April, and 855 in May. The control in the treated buildings, based on a comparison of the pre- and posttreatment counts, is shown in table 4. Mosquitoes were found in the building treated with malathion as early as the second week after spraying, but control remained at 98% to 100% for 10 weeks, then decreased rapidly. No mosquitoes were found in either of the buildings treated with Hercules AC-5727 for 10 weeks, but some were being found in both buildings by the 12th

Table 4.—Control of *Anopheles quadrimaculatus* in naturally infested buildings.

INSECTICIDE AND DOSAGE (MG./SQ. FT.)	PRE-TREATMENT COUNT (AVERAGE)	PER CENT CONTROL AT INDICATED WEEK AFTER TREATMENT												
		0	1	2	3	4	6	8	10	12	13	14	16	18
Hercules AC-5727														
100	427	100	100	100	100	100	100	100	100	98	96	99	100	100
50	52	100	100	100	100	100	100	100	100	100	0	0	0	0
Malathion														
100	231	100	100	99.6	100	98	100	98	99	87	40	0	0	0
DDT														
100	152	100	100	100	100	100	100	97	100	100	100	100	99	98

or 13th week. By the 13th week the 50-mg. treatment had completely lost its effectiveness, but the 100-mg. deposit was still highly effective and superior to malathion. There were no mosquitoes on surfaces treated with the 100 mg. of Hercules AC-5727 the 16th and 18th weeks. DDT was still giving 98% to 100% control after 18 weeks. The removal of all animals from the vicinity of this building may have reduced the number of *Anopheles* mosquitoes in the area and contributed to the control obtained.

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The Cotton Leaf Perforator and Its Control in Arizona[1]

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ABSTRACT

The cotton leaf perforator, *Bucculatrix thurberiella* Busck, is a serious pest of cotton in Arizona. Development from egg to adult averaged 18 days. Certain cultural practices intensified cotton leaf perforator injury. Predators, such as *Orius* nymphs, *Chrysopa* larvae and adults of *Collops* and *Hippodamia* were observed feeding on cotton leaf perforator larvae in the field. Adults of *Geocoris*, *Sinea*, and *Zelus*, and adults and nymphs of *Nabis* were observed feeding on cotton leaf perforator larvae in the laboratory only.

Chemical control investigations were conducted in the Yuma and Salt River Valley areas. At Yuma spray applications of insecticides were more effective than dusts. The following insecticides were found to be effective: methyl parathion, toxaphene-DDT combinations, Dilan (1 part of 1,1-bis(*p*-chlorophenyl)-2-nitropropane plus 2 parts of 1,1-bis(*p*-chlorophenyl)-2-nitrobutane), malathion, Strobane® (terpenes chlorinated to 66% chlorine content), dimethoate, SD 4402 (1,3,4,5,6,7,8,8 octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan), Sevin® (1-naphthyl methylcarbamate), Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) with DDT, Dipterex® (dimethyl (2,2,2-trichloro-1-hydroxyethyl)phosphonate) with DDT, Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) with DDT, Chlorthion® (*O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate), and a mixture of methyl parathion with endrin.

The cotton leaf perforator, *Bucculatrix thurberiella* Busck, has been a serious pest of cotton in Arizona and in the Southwest for many years (Folsom 1932, Stevenson & Kauffman 1954, Telford 1957). The larvae, in severe infestations, may skeletonize the leaves on entire plants and cause the shedding of squares, flowers and small bolls and the premature opening of older bolls, thereby lowering the grade of cotton lint (Telford 1957). Such infestations, even when finally controlled, may seriously retard the formation of new squares. Observations on the life history and natural enemies of this pest and field tests on chemical control were conducted near Yuma, Arizona in 1954 and 1955. Further field tests on chemical control were conducted in central Arizona in 1958 and 1959.

LIFE HISTORY OBSERVATIONS.[4]—These observations were based on records, taken twice daily, of cotton leaf perforator development on four, individually caged plants, each initially infested with 80 to 100 eggs of uniform age, in a field of commercially grown cotton near Yuma, Arizona, during July and August 1955. During these months the total rainfall was 1.65 inches, or 0.92 inches above normal for this irrigated desert area. Average daily maximum temperatures were 104° F. for both months. Average minimum temperatures were 76° and 79° and average daily temperatures were 90° and 92°, respectively, for July and August. The time required for the development of various stages of the cotton leaf perforator at Yuma, Arizona is summarized in table 1.

Eggs are laid singly and vertically on both surfaces of the leaves and bracts, and occasionally on immature

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[3] Agricultural Research Service, U.S.D.A.

[4] Mr. Haidar S. El Haidari cooperated in the observations at Yuma in 1955.

Table 1.—Time required for development of cotton leaf perforator. Yuma, Arizona, August 1955.

Stage	Number of Individuals Observed	Length of Stage (days)	
		Range	Average
Egg	95	4–5	4.5
Larvae:			
First instar	39	2–3	2.5
Second instar	10	1–2	1.5
Horseshoe stage	12	1–2	1.5
Third instar	6	1–2	1.5
Pupa	8	5–6	5.5

bolls. The eggs hatch in an average of 4.5 days, after which the young larvae tunnel into plant tissues at the point of egg attachment and feed internally for an average of 2.5 days before molting. Second-instar larvae migrate to the exterior, where feeding continues on either or both surfaces of leaves or bracts for approximately 1.5 days. A small, horseshoe-shaped web is then spun, usually on the lower side of a leaf. The larvae remain in the "horseshoe stage" for an average of 1.5 days during which time they molt. Third-instar larvae feed for about 1.5 days before pupating within a cocoon formed on leaves, petioles, bracts, bolls, stems, or adjacent weeds. The time required to complete the life cycle averaged 17 days in August.

In the Yuma area the practice of "stubbing" cotton has intensified the activity and injury of the cotton leaf perforator. This practice involves cutting old cotton plants near the ground level during the winter months and allowing the roots, which remain alive, to send up new stalks to produce the next season's growth. This practice results in earlier formation of squares and bolls but prevents the proper destruction of moths and immature stages of the cotton leaf perforator, which have been commonly found throughout the winter months on "stubbed" and standing stalks. At Yuma, each season since 1952, cotton leaf perforators have appeared earlier and in more destructive numbers on and near "stubbed" cotton than on cotton newly grown from seed. In 1955, for example, leaf perforator damage to foliage was common in late June in fields of stubbed cotton although similar damage was not observed until early August in another, normally planted field located several miles from the nearest stubbed cotton.

Activity of Predators.—In laboratory observations, summarized in table 2, field-collected predatory insects were individually confined in petri dishes with a single larva of the cotton leaf perforator and observed under a binocular microscope. Second-instar larvae were devoured more rapidly than older larvae. In the field, actual feeding by *Orius* nymphs, *Chrysopa* larvae and by adults of *Collops* and *Hippodamia* was observed. Predatory activity by *Geocoris*, *Nabis*, *Sinea* and *Zelus* was observed in the laboratory but not under field conditions, although individuals belonging to these genera were commonly present in the field. Their practical importance as predators of the cotton leaf perforator has not yet been demonstrated.

Table 2.—Observations on predator activity against the cotton leaf perforator. Yuma, Arizona, 1955.

Predator	Host Stage Attacked	Minutes Required to Devour Host
A. Laboratory observations (July)		
Hemiptera:		
Geocoris sp. (adult)	3rd instar larva	38
Nabis sp. (nymph)	3rd instar larva	27
(adult)	2nd instar larva	4
(adult)	3rd instar larva	32
Sinea sp. (adult)	3rd instar larva	9
Zelus sp. (adult)	2nd instar larva	4
(adult)	3rd instar larva	9
Neuroptera:		
Chrysopa sp. (larva)	2nd instar larva	5
(larva)	3rd instar larva	9
Coleoptera:		
Collops sp. (adult)	3rd instar larva	12
B. Field observations (July–August)		
Hemiptera:		
Orius sp. (nymph)	2nd instar larva	
(nymph)	horseshoe-stage larva	
Neuroptera:		
Chrysopa sp. (larva)	2nd instar larva	
(larva)	horseshoe-stage larva	
(larva)	3rd instar larva	
Coleoptera:		
Collops sp. (adult)	3rd instar larva	
Hippodamia sp. (adult)	pupa	

Chemical Control Field Tests.—Results of tests conducted in the Yuma area during 1954 and 1955 are summarized in tables 3 and 4. All insecticides were applied with power-operated ground equipment to plots of 3.3 acres in 1954 and of 1 acre in 1955, all of which were replicated four times. In the first test the insecticide dusts were applied at 25 pounds per acre whereas those dust formulations in tests 2 and 3 were applied at 20 pounds per acre (table 3). The sprays were applied with a low-volume sprayer using 10 gallons of water per acre. Table 5 summarizes results of 1958 tests at Buckeye, in central Arizona, where insecticides were applied with hand equipment at 25 pounds per acre to unreplicated 0.04-acre plots. Two 1959 tests in Pinal County, in south central Arizona, are summarized in table 6. In these tests, at Stanfield and Collidge, 5 or 6 applications of insecticides were applied by aircraft, either as sprays at 6 gallons per acre or as dusts at 30 pounds per acre, to unreplicated plots of 13.5 and 10 acres, respectively, in seasonal programs for the control of various cotton pests, including the cotton leaf perforator.

Effectiveness of the various insecticides in controlling larvae of the cotton leaf perforator was determined by counting the number surviving on cotton at the intervals shown in tables 3 to 6. At Coolidge and Stanfield records were also taken of the numbers of larvae present in the "horseshoe" stage (table 6).

Although excellent control was obtained with two dust formulations in 1954, dusts were less effective in 1955. In a test in 1955, comparing the effectiveness of several

Table 3.—Number of cotton leaf perforator larvae following single applications of several insecticides applied as dusts and sprays. Yuma, Ariz.

Insecticide (lb./gal. in sprays; per cent in dusts)	Toxicant (lb./a.)	Number of Days After Treatment					
		2	4	5	7	8	15
Test 1 (Dusts applied in July, 1954)[a]							
Toxaphene 15+DDT 5+sulfur 40	3.75+1.25+10	—	0	—	—	0	0
Endrin 2	0.5	—	6	—	—	7	13
Untreated check	—	—	486	—	—	391	268
Test 2 (Dusts applied July 13, 1955)[b]							
Endrin 2+sulfur 50	0.4+10	6.3	—	33.0	—	—	—
Toxaphene 15+DDT 5+sulfur 40	3+1+8	4.5	—	32.7	—	—	—
Chlorthion 3	0.6	3.8	—	14.5	—	—	—
Toxaphene 20+sulfur 40	4+8	6.0	—	30.3	—	—	—
Untreated check	—	10.5	—	30.5	—	—	—
Test 3 (Sprays applied July 21, 1955)[c]							
Toxaphene 6—DDT 2	2+1	—	31	—	140	—	—
Endrin 1.6	0.4	—	25	—	142	—	—
Chlorthion 4	0.6	—	23	—	55	—	—
Methyl parathion 2	0.5	—	3	—	57	—	—
Endrin 2	0.4	—	556	—	834	—	—
Endrin 2+sulfur 50	0.4+10	—	213	—	799	—	—
Toxaphene 15+DDT 5+sulfur 40	3+1+8	—	194	—	427	—	—
Dieldrin 1.5	0.3	—	194	—	538	—	—
Chlorthion 3	0.6	—	197	—	431	—	—
DDT 10+sulfur 50	2+10	—	457	—	604	—	—
Untreated check	—	—	449	—	632	—	—

[a] Larvae per 100 net sweeps.
[b] Larvae per 100 leaves.
[c] Larvae per 50 leaves.

Table 4.—Number of cotton leaf perforator larvae per 50 leaves following two applications of several insecticides. Yuma, Ariz. 1955.

Insecticide and Toxicant per Gallon (lb.)	Toxicant (lb./a.)	Number of Days After Treatment on			
		August 8			August 18
		2	4	7	25
Toxaphene 6+DDT 2	2+1	35	57	119	65
Endrin 1.6	0.3	24	43	155	204
Methyl parathion 2	0.5	4	20	14	24
Aldrin 2	0.3	19	90	381	402
Untreated check	—	149	174	319	1,914

spray and dust formulations, sprays gave considerably better control than dusts (table 3). Tests in 1955 showed that good control was obtained with sprays of toxaphene plus DDT, methyl parathion, and Chlorthion® (*O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate). In a severely infested field, spray applications of endrin, aldrin, and a combination of toxaphene with DDT were less effective than methyl parathion (table 4). These data, with those of table 3, indicate that chlorinated hydrocarbons were not as effective as organic phosphate insecticides in controlling severe infestations, and therefore should not be recommended under such conditions.

In the 1958 tests dusts containing Sevin® (1-naphthyl methylcarbamate), Dilan (1 part of 1,1-bis(*p*-chlorophenyl)-2-nitropropane plus 2 parts of 1,1-bis(*p*-chlorophenyl)-2-nitrobutane) and a mixture of malathion, DDT and sulfur gave good control of the cotton leaf perforator (table 5).

Table 5.—Number of cotton leaf perforator larvae per 100 leaves following a single application of several insecticides. Buckeye, Ariz.

Insecticide (per cent dust)	Toxicant per Acre (lb.)	Number of Days After Treatment						
		3	5	10	11	18	19	24
Test 1 (Application August 15, 1958)								
Sevin 7.5+sulfur 60	1.87+15	0	—	0	—	19	—	92
Dilan 5+sulfur 50	1.25+12.5	0	—	1	—	8	—	48
Untreated check	—	23	—	261	—	116	—	364
Test 2 (Application August 28, 1958)								
Toxaphene 15+DDT 5+sulfur 40	3.75+1.25+10	—	14	—	39	—	88	—
Malathion 5+DDT 10+sulfur 40	1.25+2.5+10	—	4	—	28	—	56	—
Dilan 5+sulfur 40	1.25+10	—	4	—	14	—	90	—
Sevin 7.5+sulfur 60	1.67+15	—	84	—	24	—	62	—
Untreated check	—	—	116	—	105	—	364	—

Table 6.—Cotton leaf perforator populations following a seasonal cotton insect control program, 1959.

Insecticide (lb./gal. in sprays; per cent in dusts)	Toxicant (lb./a.)	Average Number of Cotton Leaf Perforators per Leaf	
		Larvae	Horseshoe Stage
Test 1—Stanfield, Ariz. (5 applications from July 15–August 24)[a]			
Dusts			
Strobane 20+DDT 10+sulfur 30	6+3+9	0.7	0.9
Toxaphene 20+DDT 10+sulfur 30	6+3+9	.5	.4
Shell 4402 1	0.3	1.0	1.3
Sprays			
Shell 4402 1	.4	0.4	0.5
Toxaphene 5+DDT 2	4.6+2.3	.2	.2
Strobane 6+DDT 2	4.6+2.3	.4	.4
Test 2—Coolidge, Ariz. (6 sprays from July 21–August 27)[b]			
Dibrom 8	1	1.0	1.0
Dibrom 8+DDT 2	1 +1	0.1	0.2
Dipterex 50+DDT 2	1 +1	.1	.2
Trithion 4+DDT 2	1 +0.25	.1	.2
Dimethoate 4	1	.2	.2
Endrin 1.6+methyl+parathion 2	0.4+ .4	.2	.1
Untreated check	—	5	1.5

[a] Data taken on September 9.
[b] Data taken on September 2.

In the 1959 tests Strobane® (terpenes chlorinated to 66% chlorine content) and toxaphene, combined with DDT, gave excellent control (table 6). SD 4402 (1,3,4,5,6,7,8,8 octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan) was also effective.

Spray formulations were slightly more effective than dusts even though smaller amounts of toxicants were used per acre. In a test conducted at Coolidge, Arizona, an area where severe cotton leaf perforator infestations occur each year, all insecticide formulations tested gave commercial control of this pest (table 6), when applied in a seasonal control program for the control of lygus bugs and bollworms (*Heliothis zea* (Boddie)). Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) with DDT appeared to be more effective than Dibrom alone. Dimethoate, endrin plus methyl parathion, Trithion® (*S*-(*p*-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate) plus DDT, and Dipterex® (dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate) plus DDT (prevented cotton leaf perforators from building up to destructive numbers.

The 1959 tests showed that growers can prevent cotton leaf perforators from building up by the proper choice of insecticides which are commonly used for controlling other insects such as lygus bugs and the bollworm.

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Field Studies with Baits Against *Solenopsis saevissima* v. *richteri*, the Imported Fire Ant[1]

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Abstract

A technique based on the incorporation of dyes in food is described for determining the acceptance of baits by the imported fire ant. Results of tests with this procedure show edible oils to be very attractive to these ants. In field tests with toxic baits, Kepone® (decachloroöctahydro-1,3,4-metheno-2*H*-cyclobuta-[cd]pentalen-2-one) has been the best toxicant tested to date. Control in some plots has ranged as high as 92%. Complete eradication has not been obtained even with two treatments.

Poison baits have been used against various species of ants for many years. A classic example is the use of a sweet bait containing either sodium arsenite or thallium sulphate for the control of the Argentine ant, *Iridomyrmex humilis* Mayr. Very little work has been done to investigate the possibility of baits as a control for fire ants. Travis (1939) reported the use of thallium sulphate and thallium acetate in syrup baits against the fire ant, *Solenopsis geminata* (F.). The thallium sulphate was toxic in cage tests but not in the field, and the thallium acetate was effective in both cage and field tests; however, results in later tests were erratic, probably due to weather conditions. H. B. Green (1952) tested a bait consisting of thallium sulphate with corn meal and oil against the imported fire ant, *Solenopsis saevissima* v. *richteri* Forel, and reported the material was very effective on captive colonies but it was ignored when it was scattered in the field around the mounds. A considerable amount of unpublished work on this subject has been done by other workers, namely: Sidney B. Hayes, Department of Zoology-Entomology, Auburn University; H. B. Green, Mississippi State University and Agricultural Experiment Station; and C. C. Bowling, Texas Agricultural Experiment Station.

The purpose of this paper is to (1) describe a technique for evaluating the acceptance of baits by imported fire ant colonies and individual workers, and (2) present results of preliminary tests with toxic baits against natural infestations of the imported fire ant.

Methods and Results.—Early in our work it became evident that it would be necessary to have some standardized method for evaluating the acceptance of nontoxic and toxic baits by individual imported fire ant colonies. The following techniques have been developed.

Nontoxic-Bait Acceptance Test.—This test is easy to

[1] Presented at the meeting of the Southeastern Branch of the Entomological Society of America at Savannah, Georgia, January 1959. Accepted for publication July 14, 1960.

conduct and provides information accurate enough for most purposes. All bait materials which will dissolve or absorb an oil-soluble dye are colored with Calco-oil red or blue dye.[2] Baits which will take a water-soluble dye are colored with gentian violet or blue artificial food coloring. A concentration of 0.5% has been found to be most satisfactory. If the material to be tested is a liquid, it is poured around the base of an active mound; if the material is dry, it is sprinkled around the base of the mound. In most of the tests 150 ml. of liquid, or 50 gm. of dry bait, is used.

After a predetermined exposure period (usually 24 to 48 hours), a representative sample of the workers or other forms, if desired, is collected in vials. The ants are killed with methylene chloride and brought into the laboratory. One hundred ants are placed on a sheet of white paper, on which two identical grid patterns of 100 squares each have been printed. After the ants have been placed individually in the squares of one of the grid patterns, the paper is folded in the middle so that the grid patterns coincide. A photographic roller is used to crush the ants and force the contents of the alimentary tract onto the paper. If a dye is present in the alimentary tract it will appear as a colored stain on the paper. The number of squares stained is determined by viewing the paper over a light box.

Table 1.—Results of tests to determine the acceptance of various dyed oils and meals by imported fire ants.

Food Material	Per Cent of Workers Containing Dye After 24 Hours
Series I[a]	
Corn oil	71
Cottonseed oil	68
Fish oil	66
Peanut oil	63
Olive oil	59
Cod liver oil	56
Neat's-foot oil	43
Tung oil	0
Series II[b,c]	
Cottonseed meal and peanut oil	56
Peanut meal and peanut oil	75
Linseed oil meal and peanut oil	81
Soybean oil meal and peanut oil	67
Cottonseed meal[d]	64
Peanut meal	16
Linseed oil meal	11
Soybean oil meal	2
Refined peanut oil (standard)	65

[a] Replicated 3 times.
[b] Replicated 5 times.
[c] Where peanut oil was added, a sufficient quantity was added to saturate the meals.
[d] This meal contained natural oil.

The results of two series of tests in which this technique was employed are recorded in table 1. In the first series, eight vegetable and animal oils were compared; in the second series, four vegetable meals with and without the addition of refined peanut oil were tested. Only worker ants were examined for dye. The exposure period was 24 hours. The percentages in series I are based upon examinations of 100 worker ants and in series II on 200 ants. The tests were run in June and July when the ants were very active. The tests show that edible oils are very attractive to imported fire ants and that dried vegetable meals have little attractiveness. The cottonseed meal used in these tests contained more oil than the average and probably accounts for a higher degree of acceptance as compared with other meals. When peanut oil was added to all meals tested, a higher percentage of acceptance resulted.

Toxic-Bait Acceptance Tests.—A number of field tests were run during 1958 and 1959 to evaluate various toxic-bait formulations against natural infestations of imported fire ants. The following procedures were used in formulating the baits. The dry baits were prepared by spraying the insecticide in a methylene chloride solution on the bait as it was rotated in a small motor-driven cement mixer. In formulating the liquid baits, which were oils, the insecticide was dissolved in the oil without an auxiliary solvent. In all instances the per cent of toxicant added was calculated on the weight of the total formulation.

In all tests, pretreatment counts of active imported fire ant colonies were made on each plot. Posttreatment counts were made at regular intervals until control began to diminish.

The baits tested in 1958 consisted of dry dog food alone or in combination with pork fat, dried blood or Staley sauce bait (enzymatic protein hydrolysate). The toxicants used were dieldrin and chlordane at concentrations of 0.01% and 0.1% and heptachlor at a concentration of 0.05%. The baits were applied by hand and were scattered as evenly as possible over the plot. The plots were one-third of an acre in size and were located in pastures. The tests were run in duplicate. Further tests in this series were conducted in 1959 to evaluate heptachlor at concentrations ranging from 0.001% to 0.5% by weight in peanut oil. Neat's-foot oil and A. E. Staley Co. bait SPIB FA-19C (enzyme digested corn protein, digestion variable) were also tested as baits with 0.05% heptachlor. The materials were tested on 1-acre plots in a woodland pasture. The materials were applied with a 2-gallon compression type sprayer in narrow bands at 10-foot intervals across the plots and in a band around the entire plot. Approximately 2 gallons of bait was applied per plot. Since observations from the previous year had shown that imported fire ants would forage more than 100 feet from the mound, the plots were arranged in a checkerboard pattern so that the treated plots touched only on the corners or were separated by a buffer zone of 60 feet or more.

The results of these tests are recorded in table 2. None of the baits gave satisfactory control. The highest percentage reduction was obtained in the 1959 tests with peanut oil and heptachlor at concentrations of 0.1% to 0.5% (65% to 71% after 8 weeks). The control plots in these tests showed a 37% reduction.

In a second series of tests, Kepone® (decachloroöctahydro-1,3,4-methano-2H-cyclobuta[cd]pentalen-2-one), in concentrations of 0.1%, 0.5% and 1.0%, was evaluated

[2] Product of Pylam Products Company, Inc., 799 Greenwich Street, New York 14, New York.

Table 2.—Results of tests with some toxic baits against imported fire ants.

Bait	Toxicant	Concentration (%)	Pretreatment Count of Active Mounds	Per Cent Reduction in Active Mounds After Weeks Shown			
				1	2	4	8
		1958 tests (2 replications)					
Dog food[a]	Dieldrin	0.01	70	13	54	49	48
	Dieldrin	.1	39	33	67	31	56
	Chlordane	.01	53	0	36	38	34
	Chlordane	.1	49	35	55	38	31
	Heptachlor	.05	31	48	52	45	45
Dog food—pork fat (9:1)	Heptachlor	.05	31	41	52	26	32
Dog food—Staley sauce bait (9:1)[b]	Heptachlor	.05	42	40	79	67	50
Dog food—dried blood (19:1)	Heptachlor	.05	51	10	55	41	31
Dog food (check)	—	—	18	0	0	0	0
	—	—	37	0	22	13	11
		1959 tests (1 replication)					
Peanut oil	Heptachlor	0.5	49	24	37	59	65
		.25	31	16	61	58	71
		.1	34	32	50	71	65
		.05	66	8	38	80	36
		.025	71	8	54	62	52
		.01	32	13	56	81	50
		.001	24	0	13	17	0
Neats-foot oil	Heptachlor	0.05	16	0	0	38	6
A. E. Staley Co. bait, SPIB FA-19C	Heptachlor	.05	32	16	41	50	34
Check	—	—	46	0	9	35	37

[a] Finely ground Purina dog chow, produced by Ralston Purina Co., St. Louis, Missouri.
[b] Enzymatic protein hydrolysate, produced by A. E. Staley Manufacturing Co., Decatur, Illinois.

Table 3. Results of tests with Kepone baits againts imported fire ants.[a]

Bait	Toxicant Concentration (%)	Pretreatment Count	Per Cent Reduction in Active Mounds After Weeks Shown					
			1	2	4	8	12	18
Peanut oil	1.0	23	48	72	74	62	66	17
	.5	35	13	44	63	58	79	50
	.1	26	2	31	50	36	61	44
Peanut oil and vermiculite	1.0	38	58	71	78	85	82	37
	.5	28	13	15	42	68	86	0
	.1	25	31	31	37	76	77	0
Corn oil and vermiculite	.5	21	40	36	62	88	78	0
Corn oil and vermiculite plus 0.01% propionic acid	.5	24	55	43	70	87	92	0
Check	—	20	3	10	3	26	39	28

[a] Average of 2 replications.

as a toxicant in baits consisting of peanut oil alone; or peanut oil, corn oil, or corn oil plus propionic acid (0.01%) impregnated on vermiculite granules. Propionic acid was used because laboratory tests had indicated that it might increase the attractiveness of vegetable oils. The plot design and method of treatment was the same as in the previous test except that the granular baits were applied by hand. Twenty-three pounds of the granular baits were applied per plot.

The results of the tests with Kepone are presented in table 3. After 12 weeks there was apparently a fairly good reduction in colonies on all plots. The corn oil-propionic acid bait impregnated on vermiculite with 0.5% Kepone gave the best results (92%). However, after 18 weeks there was a great reduction in the control obtained on all plots. The majority of these mounds were very small. This fact probably indicates reinfestations from a mating flight. A heavy mating flight occurred at the time

Table 4.—Tests to determine the effectiveness of 2 successive toxic bait treatments against imported fire ants.[a]

Formulation		Pretreatment Count[b]	Per Cent Reduction in Active Mounds After Weeks Shown				
Initial Treatment	Retreatment		1	2	4	8	16
Peanut oil	Peanut oil	31	46	62	75	70	77
	Corn oil	37	59	69	53	65	51
	Peanut oil[c]	47	33	67	56	35	15
	None	31	43	74	56	47	41
Corn oil and propionic acid	Corn oil and propionic acid	19	53	79	61	68	63
	None	26	37	40	27	33	21
Peanut meal and peanut oil (5:3)	Peanut meal, linseed oil meal and peanut oil (5:5:6)	22	95	98	74	91	95
Check	—	23	0	0	0	0	0

[a] Initial treatment Aug. 28, 1959; retreatment Oct. 2, 1959. Unless otherwise indicated, the toxicant was 0.25% by weight of Kepone.
[b] Average of 2 tests.
[c] Toxicant was 0.5% Dipterex by weight.

the plots were treated. The 18-week period between plot treatment and examination is a sufficient period of time for the establishment of incipient ant colonies. The field had been disked earlier in the year and was not leveled off smoothly. This condition, when accompanied by wet, warm weather, has been found to be very favorable for survival of queens and development of the colony. In most instances, the worker ants present were very small, with only a few major workers present. It is also possible that the increase was due partly to a division of the large colonies after treatment. Imported fire ant colonies almost invariably move after they have consumed a poison bait. During the hot summer months, imported fire ants do not always build a visible mound. This is particularly true of young colonies. With the onset of cool weather, they build and increase the size of the mounds.

It is believed, however, that the main source of increase in colonies was due to reinfestation, and the results obtained through 12 weeks are a true picture of the effectiveness of the baits. The reinfestation only reemphasizes the fact that any bait treatment will require at least two or more applications to obtain complete control.

A third series of tests was conducted to determine the effectiveness of two successive bait treatments at intervals of 5 weeks. Since previous observations had indicated that the ants might develop "bait shyness," some of the plots were retreated with the same bait while in others the bait or toxicant was altered. The plot arrangement and treatment procedure were the same as in the previous tests except that the treatment bands were spaced at 20-foot intervals, and 1 gallon of liquid bait and 46 pounds of granular bait were applied per plot. The baits used in each test were as follows (unless otherwise indicated, the toxicant was Kepone at a concentration of 0.25%): (1) peanut oil, two treatments; (2) peanut oil followed by corn oil; (3) peanut oil followed by peanut oil containing 0.5% Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate); (4) peanut oil, one treatment; (5) corn oil containing 0.01% propionic acid, two treatments; (6) corn oil containing 0.01% propionic acid, one treatment; (7) peanut meal and peanut oil (5:3 ratio) followed by peanut meal, linseed meal and peanut oil (5:5:6 ratio).

The data from tests where two applications were made are given in table 4. The results show that the only bait which gave satisfactory control after 16 weeks was one consisting of peanut meal and peanut oil (95%). One application of this bait gave a high initial reduction (98% after 2 weeks) which decreased to 74% after 4 weeks.

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An Evaluation of Coding Systems for Estimating Populations of the Shaft Louse, *Menopon gallinae*[1]

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Abstract

The louse populations on 79 individual chicken hens were rated by four coding systems. The lice were then removed from each chicken and counted. The resulting numbers were subjected to several statistical tests which indicated that there were linear relationships between the numerical symbols of the coding systems and the louse infestations they arbitrarily represented. It was also found that due to improper spacing of values the accuracy of one coding system was inferior to the other three.

The need for a coding system or scale for research workers to use in determining the infestation of lice on birds is very evident when one realizes that an individual bird may be the host of a population of lice numbering several thousands. Thompson & Hosking (1957), after examining every feather except the primary wing feathers of a heavily infested chicken, reported a population of 8,270 lice. The need for a coding system is even more evident when one observes that many research workers estimate much lower total populations of lice on heavily infested birds. For example, Creighton *et al.* (1943) and Creighton *et al.* (1947) defined heavily infested birds as those harboring populations of lice ranging from 75 to 100 individuals. Fairchild & Dahm (1955), estimating entire populations of lice per bird, had estimates ranging from 32 to 400 individuals. Raun (1956) attempted to count all lice on one side of individual chickens. His counts ranged from 23 to 288 lice. Moore (1952) and Moore & Schwardt (1954) also tried to estimate the number of lice on one side of a bird and to within a 2-inch radius of the vent. They reported a heavy population as one containing more than 100 individuals. Warren, *et al.* (1948) reported a heavy infestation as 300 to 1000 lice per bird.

Edgar *et al.* (1949) stated, "It is realized of course that any method of attempting to count living lice is subject to considerable error and can be little more than an estimate. However, when a routine method of examination is used and the same operator inspects all birds, the degree of accuracy is such that comparisons between pens can be made."

Many coding systems have been used. Edgar *et al.* (1949) and Edgar & King (1950) used an eight-point system: (0) no visible lice, (1) very light, (2) light, (3) medium light, (4) medium, (5) medium heavy, (6) heavy, and (7) very heavy infestation. They observed each chicken for approximately 1 minute. Parman *et al.* (1928) used a seven-point system: No lice, very few, few, light, medium, heavy, and very heavy. Alicata *et al.* (1946) and Alicata *et al.* (1947) used a six-point system: (0) no visible lice, (1) 1 to 10 lice, (2) 10 to 100 lice, (3) 1 to 2 lice per square inch, (4) 3 to 5 lice per square inch, and (5) 6 or more lice per square inch. Moore & Schwardt (1954), Reid *et al.* (1956) and Linkfield & Reid (1958) all appeared to have used the same six-point system: (0) no visible lice, (1) 1 to 10 slight, (2) 10 to 25 light, (3) 25 to 50 medium, (4) 50 to 100 abundant, and (5) over 100 heavy. Creighton *et al.* (1943) used a five-point system: (0) no visible lice, (1) light 1 to 25, (2) medium 25 to 50, (3) heavy 50 to 75, and (4) very heavy 75 to 100 or more. Several experimenters have used four-point systems. Telford (1944) used the following system: (0) no visible lice, (Light) up to approximately 100, (Medium) proportionally more and (Heavy) proportionally more. Hamilton (1942), Warren (1945), and Telford (1947) used a system, defined as: none, light, medium, and heavy infestations. Raffensperger (1958) used the following four-point system: (0) no visible lice, (1) very difficult to find, 1 or 2 seen per bird, (2) medium infestation, and (3) heavy infestation.

None of these coding systems have been previously analyzed statistically to determine their accuracy. Therefore, the necessary linear relationship between the numerical symbols of the coding systems and the infestations of lice they arbitrarily represent could only be assumed.

Table 1.—Four coding systems used to rate louse populations.

System	Code	Cited Meaning	Interpretation
A[a]	0	No visible lice	No lice seen during visual inspection
	1	Very light infestation	1–2 lice seen during visual inspection
	2	Light infestation	Lice difficult to find
	3	Medium light infest.	Lice easily seen but thinly scattered
	4	Medium infestation	Lice more heavily scattered
	5	Medium heavy infest.	Lice numerous, many shafts of feathers appear to have several lice present
	6	Heavy infestation	Lice very numerous, most shafts appear to have one to several lice present
	7	Very heavy infest.	Every shaft appears to have several to many lice present
B[b]	0		No lice seen during a one-minute interva
	1		1–25 lice seen during a one-minute interval
	2		26–50 lice seen during a one-minute interval
	3		51–75 lice seen during a one-minute interval
	4		76–100 lice seen during a one-minute interval
	5		101–125 lice seen during a one-minute interval
C[c]	0	No visible lice	No lice seen during visual inspection
	1	Up to approx. 100	Roughly estimate a range of 1–100 lice
	2	Proportionally more	Roughly estimate a range of 101–1,000 lice
	3	Proportionally more	Roughly estimate a range of more than 1,000
D[d]	0	No visible lice	No lice seen during visual inspection
	1	Very difficult to find	1–2 lice seen during visual inspection
	2	Light-medium infest.	From 3 to rather numerous
	3	Medium-heavy infest.	Very plentiful

[a] Edgar *et al.* (1949).
[b] Developed by the authors.
[c] Telford (1944).
[d] Raffensperger (1958).

Technique.—The infestation of the shaft louse, *Menopon gallinae* (L.), was coded on 79 well-feathered white leghorn chicken hens by four coding systems, A, B, C and D as shown in table 1. No attempt was made to

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duplicate the study with other breeds of chickens or different species of lice. Each chicken was held in an upright position with one hand and the feathers were gently ruffled with the other. In this way a visual inspection was made to determine the degree of louse infestation. Each chicken was then fumigated and the feathers fluffed to remove the lice after which counts were made using a stereomicroscope (Harshbarger & Raffensperger 1959).

RESULTS.—The numbers of lice from all chickens are listed in tables 2, 3, 4 and 5, each table representing one of the coding systems tested.

An analysis of variance indicated there was a significant difference in the means of the subgroups within each coding system. The results of a multiple range test to determine which of the means of the subgroups were different are given in table 6. Those means underlined by the same straight line are not significantly different at the .05 level of probability.

The subgroups within coding systems A, B and C which were not significantly different from each other were combined and a new correlation coefficient calculated. These new values are listed under r^1 in table 7.

DISCUSSION AND CONCLUSIONS.—It was found that the linear relationship heretofore assumed between the subgroups of the coding systems and the actual numbers of lice actually existed.

This study also indicated which of the four systems tested were probably the best. Systems A, B and C were considered superior to system D because of significantly higher correlation. System A was considered inferior to systems B and C because system A contained several points not significantly different from one another. System B could be considered superior to system C because the method of determining the subgroups is less arbitrary and because there are more subgroups. System C on the other hand is much faster to use.

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Table 2.—Actual numbers of lice from birds as coded by System A.

CODE							
0	1	2	3	4	5	6	7
3	6	24	74	264	425	766	2,479
14	9	29	120	453	588	1,200	3,213
17	14	49	128	654	1,036	1,209	3,308
19	17	53	316	861	1,204	1,314	3,829
33	17	84	580	924	1,212	1,329	3,903
48	29	113	793	936	1,368	1,518	5,200
	30	130	1,205	981	1,377	2,066	5,774
	31	208		1,026	1,417	2,196	
	33	238		1,092	1,608	2,222	
	43			1,336	1,902	2,250	
	46				2,026	2,597	
	59				2,544	2,730	
					2,798	2,845	
						3,211	
						3,872	
134	330	928	3,216	8,527	19,505	31,325	27,706

Table 3.—Actual numbers of lice from birds coded by System B.

CODE					
0	1	2	3	4	5
14	3	264	425	766	2,479
17	6	453	588	1,200	3,213
19	9	580	861	1,204	3,308
48	14	654	924	1,314	3,829
	17	793	981	1,368	3,872
	17	936	1,209	1,518	3,903
	24	1,026	1,377	1,608	5,200
	29	1,036	1,902	2,196	5,774
	29	1,092	2,026	2,222	
	30	1,212	2,066	2,250	
	31	1,329	2,544	2,730	
	33	1,336	2,597	2,798	
	33	1,417		2,845	
	43			3,211	
	46				
	49				
	53				
	55				
	74				
	84				
	113				
	120				
	128				
	130				
	208				
	238				
	316				
	1,205				
98	3,137	12,128	17,500	27,230	31,578

Table 4.—Actual numbers of lice from birds coded by System C.

CODE			
0	1	2	3
3	6	425	2,479
14	9	588	3,213
17	14	654	3,308
19	17	766	3,829
33	17	861	3,903
48	24	936	5,200
	29	1,036	5,774
	29	1,092	
	30	1,200	
	31	1,204	
	33	1,209	
	43	1,212	
	46	1,314	
	49	1,329	
	53	1,368	
	55	1,377	
	74	1,417	
	84	1,518	
	113	1,608	
	120	1,902	
	128	2,026	
	130	2,066	
	208	2,196	
	238	2,222	
	264	2,250	
	316	2,544	
	453	2,597	
	580	2,730	
	793	2,798	
	924	2,845	
	981	3,211	
	1,026	3,872	
	1,205		
	1,336		
134	9,458	54,373	27,706

Table 5.—Actual numbers of lice from birds coded by System D.

CODE			
0	1	2	3
3	6	24	425
14	9	29	588
17	14	49	766
19	17	53	1,036
33	17	74	1,200
48	29	84	1,204
	30	113	1,209
	31	120	1,212
	33	128	1,314
	43	130	1,329
	46	208	1,368
	55	238	1,377
		264	1,417
		316	1,518
		453	1,608
		580	1,902
		654	2,026
		793	2,066
		861	2,196
		924	2,222
		936	2,250
		981	2,479
		1,026	2,544
		1,092	2,597
		1,205	2,730
		1,336	2,798
			2,845
			3,211
			3,213
			3,308
			3,829
			3,872
			3,903
			5,200
			5,774
134	330	12,671	78,536

Table 6.—The ranked means[a] of subgroups for the four coding systems.

SYSTEM	0	1	2	3	4	5	6	7
A	22	28	103	459	853	1,500	2,088	3,958
B	25	112	933	1,458	1,945	3,947		
C	22	278	1,699	3,958				
D	22	28	487	2,243				

[a] Means underlined with the same straight line are not significantly different at the .05 level.

Table 7.—Correlation coefficients for the coding systems.

SYSTEM	r	r'
A	0.82	0.87
B	.86	.87
C	.81	.85
D	.68	

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Effect of Migration, Temperature, and Precipitation on Thrips Infestations in South Texas[1]

James A. Harding[2]

Abstract

A two-year study on onion thrips (*Thrips tabaci* Lind.) yielded data which indicated that concentrated migrations of thrips into onion fields do not occur in South Texas. Thrips movement into onion fields lessened as other host plants became lush and onions approached maturity. Precipitation and mean daily temperatures below 50 degrees F. reduced thrips movements. Driving rains reduced thrips infestations on the plants and mean daily temperatures above 58 degrees F. favored destructive infestations. Heavy infestations resulted from build-up in the field and not from thrips movement into the field.

Previous work in South Texas by Chapman *et al.* (1945, 1947), Fife *et al.* (1948), Hibbs & Ewart (1946), Mayeux & Wene (1950), Richardson (1952, 1953, 1954, 1957), Richardson & Wene (1952, 1955, 1956), Wene (1948), Wene & Mayeux (1949) and Wene & White (1952) on thrips has been voluminous, but has dealt only with insecticidal control studies. The present study was initiated to secure information on the more basic aspects relating to thrips attacking onions in South Texas. Results of weekly trapping, infestation, and weather data throughout two growing seasons yielded information on the relationships of these factors that may be general knowledge to some but has never been recorded for this area.

This study was suggested by the data presented in papers by Boyce & Miller (1953), Horsfall & Fenton (1922), Shirk (1951), and Wolfenbarger & Hibbs (1958), which showed that huge migrations of onion thrips occurred from alfalfa, red clover, wheat, and oats to other host plants when these crops were harvested. Similar results were obtained by Wilcox & Howland (1950). This phenomenon was strikingly represented in a graph by Faulkner (1954) between alfalfa cutting and cotton infestations. Gaines (1934) and Hightower & Martin (1956) studied migration and dispersion of thrips in cotton showing the times of greatest movements.

Methods.—Tanglefoot traps consisting of a $4\frac{1}{2}$- $\times 5\frac{1}{2}$-inch cardboard mounted on a 5-×8-inch piece of plywood were located around and in a rectangular acre of transplanted onions following the first irrigation. During the 1958–59 season, eight locations on the perimeter of the block were used. Two traps were set side by side at each of the following points, N., NE., ESE., SES., S., SW., W., and NW. One series of traps was 2 feet and the other $4\frac{1}{2}$ feet above the ground level. In the 1959–60 season study, five traps 2 feet above the ground level were arranged at SW., SE., NE., and NW. points around a central trap in the middle of the field. Each trap was at least 150 feet from any other trap with two of the traps on the perimeter of the field and three traps within the field.

Weekly counts of the number of thrips on the traps were taken and the $4\frac{1}{2}$-×$5\frac{1}{2}$-inch card was then replaced or cleaned. At this same time the number of thrips per 30 to 70 plants selected at random over the block was recorded. This would explore the relationship of flights to plant infestation. Mean daily temperatures for the preceding week, and the total precipitation for the week preceding the thrips records, were obtained from the official weather station instruments located at a maximum of three-tenths mile from the block under observation.

Results and Discussion.—The following facts became apparent during the course of this study. The predominant species of thrips on onions collected during both seasons was *Frankliniella occidentalis* (Pergande). No differences due to height of the traps above the surface of the soil appeared during the 1958–59 season. No indications of a particular pattern of thrips movement within the fields could be detected during the 1959–60 season.

Fig. 1.—A comparison of thrips flights with onion plant infestation, 1958–59 season.

Figures 1 and 2 illustrate graphically the relationships of thrips migration to plant infestation during the 1958–59 and 1959–60 growing seasons, respectively. The concentrated migrations of thrips from one crop to another as a result of cutting or harvesting of other hosts were not evidenced in these studies perhaps, because no large acreages of suspected infestation sources were harvested. The data indicate that as other favorable host plants

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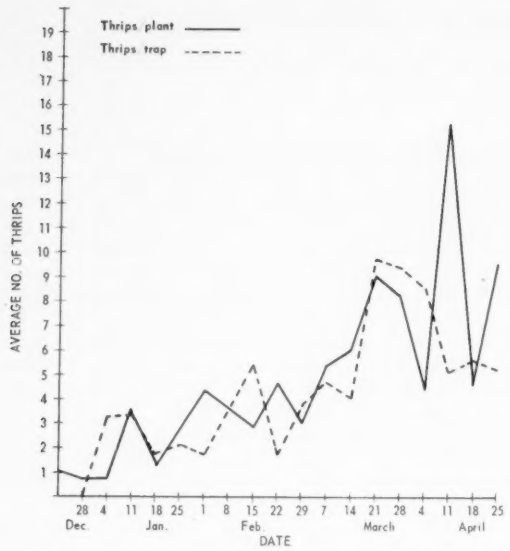

FIG. 2.—A comparison of thrips flights with onion plant infestation, 1959–60 season.

begin to grow and become abundant the thrips movement into onions lessens. Flights in onion fields decreased and continued to do so after the first week in April.

A few comparisons of trapped thrips with the weather are shown in table 1 and show some other relationships and bring out some factors that could not be included in the table. In figure 1, an increase in the number of trapped thrips may be noted on January 26, February 9, and March 16, and decreases on February 2 and 23. The week preceding January 26 compares favorably with that of February 23 weatherwise; however, January 26 precipitation came as drizzles in 3 days leaving 4 days favorable for thrips movement while February 23 precipitation fell over 5 days leaving only 2 days favorable for movement and 0.22 inch of this precipitation fell as a rain. Increases of trapped thrips on February 9 and March 16 are attributed to mean temperatures above 50 degrees F. and little or no precipitation. The sharp decline in trapped thrips shown for February 2 was caused by the cool temperatures and 0.48 inch of the 0.62 inch of moisture which fell as a windy rain. These phenomena follow the same basic pattern in figure 2 and are responsible, in part, for variations in thrips movement.

Table 1.—Mean temperatures and total precipitation for the week ending on the date indicated taken during two onion-growing seasons.

1958–59 DATE	MEAN TEMPERATURE (F.)	PRECIPITATION (INCHES)	1959–60 DATE	MEAN TEMPERATURE (F.)	PRECIPITATION (INCHES)
Dec. 29	54.5	0.55	Dec. 28	57.4	Trace
Jan. 5	39.8	0.27	Jan. 4	52.5	0.25
Jan. 12	46.8	0.23	Jan. 11	51.1	0.42
Jan. 19	55.0	0.01	Jan. 18	60.9	0.29
Jan. 26	53.7	0.25	Jan. 25	40.9	Trace
Feb. 2	48.2	0.62	Feb. 1	54.2	0.01
Feb. 9	51.0	0.04	Feb. 8	57.5	0.71
Feb. 16	59.1	0.34	Feb. 15	56.3	0.69
Feb. 23	53.6	0.37	Feb. 22	52.3	0.00
Mar. 2	58.9	0.11	Feb. 29	50.0	0.04
Mar. 9	58.0	0.00	Mar. 7	44.2	0.16
Mar. 16	61.6	0.01	Mar. 14	55.1	0.12
Mar. 23	58.0	0.00	Mar. 21	58.2	0.02
Mar. 30	67.2	0.00	Mar. 28	66.7	Trace
Apr. 6	70.2	0.00	Apr. 4	68.1	0.40
Apr. 13	60.6	0.63	Apr. 11	70.5	0.00
Apr. 20	69.5	0.11	Apr. 18	74.6	0.44
Apr. 27	69.1	0.14	Apr. 25	74.2	Trace

Thrips per plant as related to the weather may be seen by comparing figures 1 and 2 with table 1. The data indicate that cool temperatures or rains are detrimental to an infestation on the plants. Thrips infestations started increasing on the plants on February 2, and reached a peak on February 13 in 1959. This period had a mean daily temperature above 50 degrees F. and was relatively dry. Between February 13 and 23, 0.71 inch of rain fell over 2 days and reduced the infestation. The reduction in the plant infestation that appeared after March 16, 1959 is attributed to an abundance of other favorable host plants in the area. In 1960 an example of the effect of favorable temperatures on thrips increase and the effect of 0.44 inch of rain on thrips reduction occurred. There were 15.3 thrips per plant on April 11, and after a cold rain only 4.5 thrips remained by April 18. In 1960 the thrips stayed on the onion plants. This fact was attributed to the slow growth of other host plants because of a late spring, in contrast to the previous season.

Plant infestation is correlated with thrips migration until other factors begin to dominate the picture. Both counts go up and down in relation to each other until temperatures allow the reproductive capacities of the thrips to become more efficient. During both seasons of these studies, when infestations on the plants became very heavy, trapping records showed that thrips migrations became static or decreased slightly. This would indicate that destructive infestations of thrips develop primarily from build-up by breeding within the field and secondarily from the migrations of these insects.

Some relationships indicated by this study for South Texas are: No concentrated movement of thrips into onion fields occurred. Thrips movement into onions lessened as other host plants became abundant and the onions approached maturity. Precipitation and mean daily temperatures below 50 degrees F. reduced thrips movements. Hard rains reduced thrips infestations on the plants and mean daily temperatures above 58 degrees F. favored heavy infestations. Destructive infestations resulted from build-up by breeding in the field and not from thrips movement from outside of the field into the field.

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Comparison of Ronnel, DOWCO 109, and DOWCO 105 for Systemic Control of Cattle Grubs in Alberta[1]

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ABSTRACT

Comparative treatment of separate groups of cattle for the systemic control of cattle grubs (*Hypoderma bovis* (L.) and *H. lineatum* (De Vill.)) showed that s/c (subcutaneous) injection of Dowco 109 (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidothioate) emulsion at 15 mg./kg. and oral drench of Dowco 105 (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl ethylphosphoramidothioate) wettable powder at 25 mg./kg. were more effective than ronnel (Trolene®) boluses at 110 mg./kg. against the internal and hypodermal grubs, respectively. S/c injection of Dowco 109 solution at 5 mg./kg. and Dowco 105 emulsion at 25 mg./kg., and topline spray of Dowco 109 wettable powder at 0.75% were not effective. Other treatments with s/c and IM (intramuscular) injections of Dowco 109, oral drenches of Dowco 109 and Dowco 105, and feed additives of Dowco 109 were as effective as ronnel.

Lack of control in a few cattle that received IM injection and feed additive treatments of Dowco 109 was attributed to insecticidal build-up that was slower than degradation. With these treatments a few cattle would provide reinfestations that might frustrate areal eradication of the grubs.

The s/c injections resulted in sterile abscesses and fibrous lumps at the site of injection. Ataxia was seen in some of the cattle treated with ronnel and with Dowco 109 s/c at 15 mg./kg., contrary to a reported wide interval between the larvicidal and toxic levels of Dowco 109. The symptoms might have been a by-product of larval death and disintegration, which suggests that uninfested cattle be used in assessing the mammalian toxicity of larvicidal systemic insecticides.

Treatment of cattle with ronnel (Trolene®) boluses at 100 to 110 mg./kg. has produced good control of the internal grubs of *Hypoderma bovis* (L.) and *H. lineatum* (De Vill.) in Canada (Weintraub *et al.* 1959, a and b) but general use has emphasized its disadvantages of laborious treatment, regurgitated boluses and narrow margin of safety to the cattle. Daily low-level treatment of cattle with ronnel as a feed additive (Weintraub *et al.* 1959b) was a promising solution to these problems for producers with proper feeding facilities. However, on most ranches and farms in Alberta calves are fed only intermittently to tide them over short periods of severe weather. Therefore, the method is limited mainly to feedlot cattle.

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C. O. M. Thompson resigned in August 1959.

Two new compounds, Dowco 109 (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidothioate) and Dowco 105 (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl ethylphosphoramidothioate), seemed to offer advantages of safety and versatility which were examined experimentally in 1957 and 1958. Preliminary evidence (Crenshaw 1957) from small-scale tests indicated that Dowco 109 and Dowco 105 oral drench controlled grubs of *H. lineatum* at 20 and 25 to 50 mg./kg., whereas the cattle tolerated doses of 40 and 150 mg./kg., respectively, without exhibiting symptoms of toxicity. The low effective dosages rendered the new compounds feasible for parenteral use. In addition, they were potentially more useful in chronic low-level treatment as feed additives because the duration of feeding and the daily amounts could be reduced considerably from those required in ronnel therapy.

It was our purpose to compare the two new compounds as single oral drenches, injections, sprays, and feed additives. Their effectiveness was evaluated against ronnel boluses at the recommended dosage of 110 mg./kg.

MATERIALS AND METHODS.—In each year groups of beef calves were used from a single herd that had been weaned in November. The calves were allotted randomly to the various treatment groups in samples of 15 in 1957 and 20 in 1958.

In 1957 the test cattle were brought to Lethbridge in mid-February for treatment and observation, at which time they carried an average of 20.7 (range 0 to 72) grubs in the warbles. When given the insecticides on February 14, they were enduring an outbreak of shipping fever and coccidiosis, for which they were treated at the same time. The doses of insecticides were based on the measured weights of the individual calves, accurate to the nearest 5 pounds. The single oral treatments consisted of ronnel boluses containing 40% active ingredient and aqueous suspensions of wettable powders of 25% Dowco 109 and Dowco 105. The subcutaneous injectables of Dowco 109 consisted of an emulsion and a solution containing 100 mg./ml. active ingredient; that of Dowco 105 was an emulsion containing 300 mg./ml. (table 1).

In 1958 the calves were treated and observed on the ranch. Ronnel boluses and Dowco 109 feed additives were given commencing December 3, 1957. The Dowco 109 spray was administered on January 5 and the Dowco 109 subcutaneous and intramuscular injections on January 23, 1958. Only the cattle in the last two groups contained hypodermal grubs at the time of treatment (table 1). The Dowco 109 subcutaneous and intramuscular injectables were emulsions containing 250 mg./ml. The insecticidal dosage of each was 15 mg./kg. (range 12 to 18 mg./kg.), based on the average measured weights of the calves in the respective groups. The Dowco 109 wetable powder spray was a 0.75% aqueous suspension of 25% material, delivered from a cone nozzle to the backs and upper parts of the sides in amounts of 1 gallon per calf at 400 p.s.i. The feed additives were in the form of reprocessed wheat screenings containing 10% Dowco

Table 1.—Summary of insecticide treatments administered to groups of calves for systemic control of internal cattle grubs (15 calves per group in 1957 and 20 in 1958). Lethbridge, Alberta.

TREATMENT GROUPS	DOSAGE (MG./KG.)	NO. GRUBS					
		Range	Mean[a]	Transformed Mean[b]	S.E.	DERIVED MEAN[c]	PER CENT CONTROL[d]
		1957					
1. Untreated	—	0–155	49.5	1.40	0.169	24.4	—
2. Ronnel bolus, oral	110	0– 29	4.6	0.15	0.112	0.5	98
3. Dowco 109 W.P., oral drench	10	0– 5	0.7	0.16	0.059	0.5	98
4. Dowco 109 W.P., oral drench	15	0– 3	0.5	0.11	0.051	0.3	99
5. Dowco 105 W.P., oral drench	20	0– 4	1.3	0.35	0.026	1.3	95
6. Dowco 105 W.P., oral drench	25	0– 6	1.0	0.17	0.074	0.5	98
7. Dowco 109 emul., s/c inject.	10	0– 9	0.9	0.14	0.075	0.4	98
8. Dowco 109 sol., s/c inject.	5	1–129	49.3	1.53	0.119	32.6	—
9. Dowco 105 emul., s/c inject.	25	5– 66	25.4	1.32	0.081	19.7	19
		1958					
10. Untreated	—	14–157	59.6	1.66	0.080	44.3	—
11. Ronnel bolus, oral	110	0– 36	11.9	0.90	0.113	7.0	84
12. Dowco 109 emul., s/c inject.	15	0– 17	1.9	0.26	0.084	0.8	98[e]
13. Dowco 109 emul., I.M. inject.	15	0–104	23.6	0.86	0.154	6.2	81[e]
14. Dowco 109 W.P., topline spray, 0.75%		2– 96	43.9	1.49	0.108	29.6	33
15. Dowco 109 feed additive, 22 days	0.5	0– 53	8.5	0.59	0.136	2.9	94
16. Dowco 109 feed additive, 44 days	0.5	0–110	15.2	0.53	0.165	2.3	95
17. Dowco 109 feed additive, 11 days	1.0	0–142	29.1	0.91	0.170	7.1	84
18. Dowco 109 feed additive, 22 days	1.0	0– 88	17.8	0.58	0.179	2.8	94

Significance:
1957: Gp. 1>Gps. 2, 3, 4, 5, 6, 7 (P<.01); Gp. 1 vs. Gps. 8, 9 (P>.05). Gp. 2 vs. Gps. 3, 4, 5, 6, 7 (P>.05); Gp. 2<Gps. 8, 9 (P<.01). Gp. 3 vs. 4, Gp. 5 vs. 6, Gp. 3 vs. 7 (P>.05).
1958: Gp. 10>Gps. 11, 12, 13, 15, 16, 17, 18 (P<.01); Gp. 10 vs. Gp. 14 (P>.05). Gp. 11 vs. Gps. 15, 16, 17, 18 (P>.05); Gp. 11>Gp. 12 (P<.01); Gp. 11 vs. Gp. 13 (P>.05). Among Gps. 15, 16, 17, 18 (P>.05). Gp. 12<Gp. 13, Gp. 13<Gp. 14 (P<.01).

[a] Exclusive of 20.7, 5.6 and 5.8 grubs per calf that cut the skin before the time of treatment in Gps. 1–9, 12 and 13, respectively.
[b] Transformation was log $(x+1)$.
[c] Mean, derived back from mean of transformed data, *i.e.*, antilog $(\bar{x})-1$.
[d] Based on derived means.
[e] Based on 38.1 grubs in the controls (Gp. 10), which was the number that cut the skin after the time of treatment.

109 which were incorporated at the mill into protein concentrate calf pellets at two levels, 78 and 156 p.p.m. Dowco 109, corresponding to the respective daily levels of 0.5 and 1.0 mg./kg. (table 1). The pellets were distributed once daily to the segregated groups of calves in amounts of 2.5 pounds per calf. The two groups that were fed for 22 days (table 1) were watched daily for signs of inappetence or failure of some calves to compete at the trough.

In each year the numbers of grubs were assessed by squeezing out all grubs reaching the back each week, beginning 1 week after the time of treatment and ending when three consecutive examinations of the whole herd showed no grubs. The effectiveness of internal grub control was calculated as a percentage comparison of the cumulative number of grubs reaching the backs in the treated and untreated cattle after the date of treatment. The effectiveness of hypodermal grub control was assessed only in 1957. It was calculated as a percentage of the number of dead grubs over the total squeezed out in each group at the first squeezing.

Statistical analysis was complicated by the marked skewness of the distributions, which was somewhat reduced by applying a log $(x+1)$ transformation. However, the variances still showed significant heterogeneity by Bartlett's test (Snedecor 1956, table 10.20.1). Therefore, individual t-tests were applied for those comparisons of interest where the variances could be combined by reference to two-tailed variance ratio tables (Snedecor 1956, table 4.8.1), and Behrens-Fisher tests (Fisher & Yates 1953, table V_1) were used for the rest.

RESULTS AND DISCUSSION.—*Oral Treatments.*—Table 1 shows that the new compounds were as effective as ronnel against the internal grubs (table 1, groups 3, 4, 5, 6). Evidently, too, doses of 10 or 15 mg./kg. of Dowco 109 and 20 or 25 mg./kg. of Dowco 105 were at, or above, the maximal level for control. This was confirmed for Dowco 109 by Drummond & Graham (1959), who obtained 92% and 94% mortality at 15 and 25 mg./kg., respectively, which are comparable to the 98% and 99% at 10 and 15 mg./kg. found here. Turner & Gaines (1958), DeFoliart *et al.* (1958), and Rich & Ireland (1959) obtained similar mortalities with Dowco 109 oral doses of 15 mg./kg.

The new compounds appeared to be more effective than ronnel against the combined instars of the hypodermal grubs (table 2), which were all *H. lineatum*. *H. bovis* did not cut the skin until 2 weeks later. Further analysis showed that, as in ronnel treatment, larvae in the third instar were more resistant than those in the first and second (Weintraub *et al.* 1959a). In the final analysis, however, only Dowco 105 at 25 mg./kg. showed a marked improvement over ronnel against the resistant third-instar larvae (table 2). However, Roth & Eddy (1959) found that Dowco 109 was more effective than ronnel against large third-instar larvae.

Injections and Spray.—In 1957 the subcutaneous injection of Dowco 109 emulsion at 10 mg./kg. was as effective as ronnel against both the internal and hypodermal grubs and in 1958, at 15 mg./kg., it was significantly more effective (table 1, groups 7 and 12; table 2, group 7). In 1957 the injected Dowco 109 solution and Dowco 105 emulsion were totally without effect in controlling both phases of the grubs (tables 1 and 2, groups 8, 9). In 1958 the intramuscular injection of Dowco 109 (table 1, group 13) did not give significantly different control from that produced by the ronnel boluses. However, the injected group contained six animals with 20 to 104 grubs, as compared with an average of 6.4 grubs in the remaining 14 animals. Drummond & Graham (1959) found that the same dose of Dowco 109, injected intramuscularly, produced a control of 76%, which is comparable to the present 81% (table 1, group 13).

Table 2.—Effect of ronnel, Dowco 109, and Dowco 105 on the hypodermal larvae of *H. lineatum*, present at the time of treatment. Lethbridge, Alberta, 1957.

GROUPS	GRUBS SQUEEZED OUT[a]		PER CENT DEAD		
	Mean	Instar III (%)	Total Instars	Instars I and II	Instar III
1. Untreated	18.1	40	1	0	2
2. Ronnel	24.2	44	67	87	37
3. Dowco 109 10 mg./kg.	20.0	20	85	98	32
4. Dowco 109 15 mg./kg.	18.9	32	83	97	53
5. Dowco 105 20 mg./kg.	20.8	39	76	91	51
6. Dowco 105 25 mg./kg.	23.3	34	93	98	82
7. Dowco 109 s/c 10 mg./kg.	20.3	32	74	89	43
8. Dowco 109 s/c 5 mg./kg.	20.6	36	6	6	5
9. Dowco 105 25 mg./kg.	20.2	39	24	28	17
Mean	20.7	35			

[a] Grubs squeezed out 1 week after treatment; grubs that appeared in the backs after treatment were not included.

In the practical comparison of intramuscular and subcutaneous injections of Dowco 109 higher doses of the former are required to equal the effect of lower doses of the latter. This is illustrated in figure 1, which shows a dosage-mortality curve from the data on intramuscular injections by Drummond & Graham (1959), with comparable points from both intramuscular and subcutaneous injections in the present study. The maximal effect of the intramuscular treatment appears to be at about 25 mg./kg. and seems to be equal to the effect of either the 10 or 15 mg./kg. subcutaneous dose.

The effectively larvicidal Dowco 109 emulsion, given subcutaneously at 10 or 15 mg./kg., caused inflammation of the subcutaneous tissues at the site of injection in a few cattle. The inflammation disappeared in about 4 to 14 days. In contrast, both the Dowco 109 subcutaneous solution and Dowco 105 emulsion caused severe and long-lasting sterile abscesses, some of which had to be lanced while others changed to hard fibrous masses that had to be excised. It seems likely that the formulation, nature, and quantity of the materials were responsible for irritating the tissues and producing local reactions that possibly prevented the systemic dispersion of the compound within the animal. No local reactions were detected in the cattle that had been injected intramuscularly.

The Dowco 109 spray had no significant effect on the number of grubs (table 1, group 14). At similar concentrations and amounts of materials, but with complete coverage of the cattle, Roth & Eddy (1959) obtained 71% to 100% control and Howell *et al.* (1958) obtained 77% to 95%. Howell *et al.* also found that a topline spray at double the concentration was equally effective. Drummond & Graham (1959) obtained good control with a complete cover spray at 1% concentration but not at

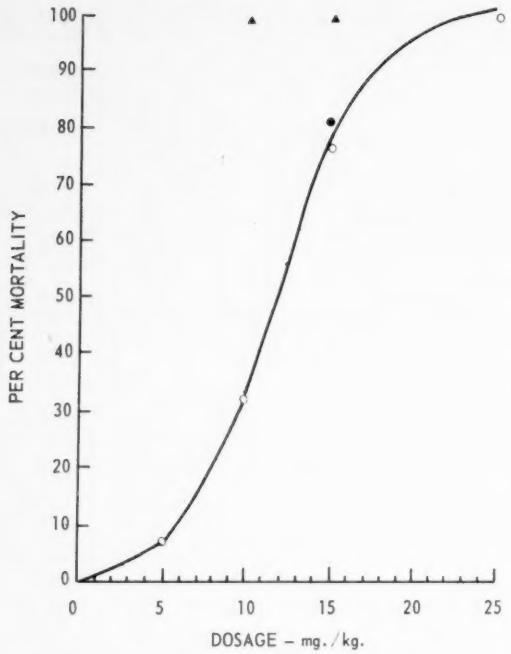

FIG. 1.—Dosage-mortality curve for Dowco 109 intramuscular (IM) injection against internal cattle grubs, with comparable points for Dowco 109 subcutaneous (s/c) injection.
○—IM—from data of Drummond & Graham (1959).
●—IM—from present study.
▲—s/c—from present study.

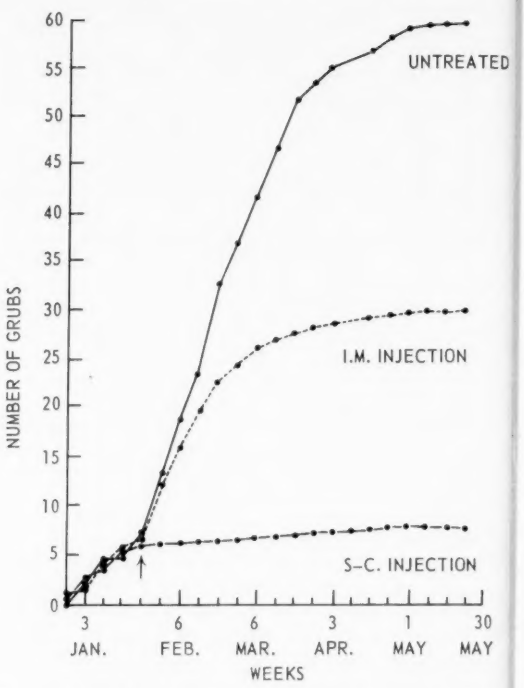

FIG. 2.—Cumulative average numbers of grubs per calf arriving in the backs of three groups of 20 calves, indicating differences in the rate of mortality resulting from treatments with Dowco 109 intramuscular and subcutaneous injections at 15 mg./kg. Arrow shows date of treatment, January 23, 1958.

0.5%. Turner & Gaines (1958) found that a complete cover spray at 1% gave about 90% control. DeFoliart *et al.* (1958) considered that complete cover sprays at 0.25% and 0.5% were mostly inadequate, although one group at 0.5% showed a control of 80%. It seems that such factors as the amount of insecticide, the extent of coverage, and penetration of winter coat played an important part in preventing control of the grubs. In the present study, collection of the runoff from a few of the sprayed cattle showed that about 50% to 60% of the material was shed by the long thick hair of the cattle.

Feed Additives.—The low-level treatments were equal in effectiveness to the ronnel bolus treatment and to each other, despite the apparent differences among the untransformed or original means (table 1, groups 11, 15, 16, 17, 18). Also, there was no significant difference among the two daily dosage levels, two total dosages, and three durations of treatments tested. In each of the groups a few of the animals produced disproportionately large numbers of grubs. Thus, Group 15 had four animals with 19 to 53 grubs, Group 16 had four with 26 to 110 grubs, Group 17 had six with 30 to 142 grubs, and Group 18 had five with 34 to 88 grubs. DeFoliart *et al.* (1958) also found a similar situation resulting from his Dowco 109 feed additive treatments and concluded that some of the cattle consumed too little feed to dose themselves adequately. Our observations on two groups over the entire feeding period showed that none of the cattle received sub-standard doses by inability to compete at the feed trough or through extreme inappetence, though their normal feed consumption might have varied widely. It was also thought that the pelleting process might have caused variable deterioration of the incorporated insecticide but analysis (Getzendaner 1958) of the active ingredient in samples taken from surplus pellets 1 year after the time of treatment showed no such deterioration. However, these possibilities appear to be of little consequence since the group that was injected intramuscularly with Dowco 109 (table 1, group 13) showed a great variation in grub survival that was not correlated with the range of dosage (12 to 18 mg./kg.) Therefore, some explanation other than the effects of variable treatment is required.

Figure 2 indicates that the intramuscular injection of Dowco 109 does not show appreciable effectiveness until several weeks after treatment whereas the subcutaneous injection is effective immediately. Similarly, low-level treatment with ronnel showed a delayed effect in comparison with the single full-strength bolus treatment (Weintraub *et al.* 1959b). Similar comparisons could not be made with the Dowco 109 low-level treatments because they were administered too early in the season to obtain the type of data that would permit graphing the rate of popu-

lation suppression.

It is suggested that the low-level treatment and the intramuscular injection are both characterized by the *slow rate* at which the toxic components build up to the level that is effective against the grubs. In the former this would take place through gradual consumption of small doses, and in the latter through gradual absorption of the insecticide from a depot in the tissues, similar to that of emulsified drugs that have been injected intramuscularly (Musser & Bird 1958). If, through natural variability, the rate of metabolization or degradation of the insecticide should outstrip that of the gradual buildup in a few of the cattle, the dosage would not be maintained at a sufficiently high level for effective grub mortality. The same situation could exist to a lesser degree with the faster-acting oral or subcutaneous treatments. For example, two animals that were treated with ronnel produced 36 grubs and one that was treated subcutaneously with Dowco 109 at 15 mg./kg. produced 17 grubs (table 1, groups 11 and 12). However, the probability of encountering "escapes" in a group of cattle would generally increase for methods of treatment that feature a slowly paced build-up of the insecticide, and still further increase through variable consumption of the insecticide as in the free-choice feeding method. Since the detection of "escapes" by grub assessment depends entirely on whether they are heavily infested, they probably have been overlooked in experiments on lightly infested cattle.

Table 1 shows the difference in means brought about by transforming the data for stabilizing the variances preparatory to analysis. Transformation minimizes the outlying values in the range of grub infestation, and is necessary for adequate comparison of insecticidal effectiveness in groups of cattle with such large variations. However, the potential biological consequences of the outlying values cannot be minimized similarly. First, the immediate benefits of treatment would be denied the producer for some animals in the herd treated by methods that permitted some "escapes." More important, the presence of such animals in localized spots in an area, in which an eradication program by thorough treatment is in force, would tend to frustrate the achievement of eradication. It has been estimated that an over-all mortality of 95% of the grubs is required to make permanent reductions in the grub populations (Kühl 1958). Thus, the few animals harboring large numbers of grubs might constitute foci for massive reinfestation.

Symptoms of Toxicity.—Toxic symptoms were not observed in the cattle in 1957, even though they were sick with shipping fever and coccidiosis. Differences in severity and duration of the infections could not be detected between the untreated control group and the various treated groups. In 1958 toxic symptoms, of the kind termed ataxia (Weintraub *et al.* 1959a and b), appeared in 14 of the animals treated with ronnel and in 10 of those injected subcutaneously with Dowco 109 at 15 mg./kg. The symptoms varied from a slight stumbling gait in most of the cattle to extreme incoordination in three belonging to the Dowco 109 group. None of the animals were treated with specific antidotes for organophosphate poisoning, but the condition cleared up after they were forced to exercise.

It has been reported that oral drenches of Dowco 109 did not produce toxic symptoms in cattle at 50 (McGregor *et al.* 1958) or 100 mg./kg. (Drummond & Graham 1959). Oral drenches of Dowco 105 at 120 to 130 mg./kg. produced slight symptoms of toxicity (Baker *et al.* 1959). By comparison the effective and toxic dosages for ronnel were 100 and 125 mg./kg., respectively (Radeleff & Woodard 1957).

The occurrence of ataxia among the cattle injected subcutaneously with Dowco 109 in 1958, and similar reports about cattle treated orally (Rich & Ireland 1959, DeFoliart *et al.* 1958), would seem to nullify the estimate of its superior margin of safety as compared with that of ronnel. In extenuation, none of the treatments resulted in toxic symptoms in 1957, even though the cattle were under considerable stress through shipping fever and coccidiosis when treated. In addition, the symptoms have not been reported from several other experiments in which Dowco 109 was given orally and intramuscularly at levels between 10 and 25 mg./kg. (Turner & Gaines 1958, Howell *et al.* 1958, French *et al.* 1958, Roth & Eddy 1959, McGregor *et al.* 1958, Drummond & Graham 1959). Thus the question of the superior safety of Dowco 109, and to a lesser degree Dowco 105, is still in doubt. It has been suggested that the symptoms of toxicity seen after treatment with either ronnel or these newer compounds are a side effect resulting from the death of the larvae (Peterson & Kingscote 1959, Rich & Ireland 1959). If so, their occurrence would be a corollary of the larvicidal efficiency of the insecticide used, and might be influenced not by the degree of overdosing but by such factors as the number of grubs present in an animal, their species composition, the timing of the treatment, and the rates at which the grub population dies and grubs disintegrate. This might have been the case in the present studies, but the question of anaphylactic shock involvement urgently requires further investigations. In the meantime, estimates of chemical toxicity of cattle systemics should perhaps be based upon treatments of animals that are uninfested.

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Artificial Infestations of the Northern Cattle Grub, *Hypoderma bovis*, in Texas[1]

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ABSTRACT

Adults of *Hypoderma bovis* (L.) were reared in the laboratory at Kerrville, Texas, from grubs that had dropped from imported cattle. The flies were induced to mate and oviposit by tethered flight, thus infesting calves with four replicates of 50, 100, 200, 500 and 1,000 eggs in April and May. The outdoor behavior of the flies in the mating and oviposition procedures was similar to that observed in Canada, where *H. bovis* is indigenous. However, air temperatures above 85 to 90° F. curtailed mating and oviposition, which could therefore be induced only in the mornings and evenings. Survival of the larvae to the hypodermal stage was density-related, taking the form $Y = 5.07X^{.280}$ for egg densities up to 500 per animal, which was a similar relationship to that obtained in Canada. However, there was a markedly lower survival in the late-season 1,000-egg infestations. This was attributed to either infertility or mortality of the eggs because of the excessively high temperatures. It was concluded that climatic factors at Kerrville did not limit the normal survival of the larvae but partly limited the reproductive activity of the adults and affected the viability of the eggs. Reasons for the absence of *H. bovis* at Kerrville might be determined through studies of the survival and development of puparia under natural conditions.

The northern cattle grub, *Hypoderma bovis* (L.), has not become established in Texas even though infested cattle have been imported frequently from the northern areas of the United States and Canada where this species is indigenous (Bishopp *et al.* 1949). For example, infested cattle have had to be acquired from South Dakota for experiments on *H. bovis* at the Kerrville, Texas, Laboratory, Entomology Research Division, U. S. Department of Agriculture (McGregor & Bushland 1957). The workers at that laboratory wished to use the recently developed methods of inducing mating and oviposition of cattle grubs (Weintraub 1961) to infest cattle with known numbers of grubs of *H. bovis* for screening insecticides. Therefore, through a cooperative arrangement between the government departments of the United States and Canada, the senior author demonstrated those methods and cooperated in the early work of these jointly planned experiments at Kerrville in the spring of 1956. The other authors did the main work of rearing the adult flies, carrying out the mating and infesting procedures (W.S.M.) and assessing the final results of the infestations (H.M.B.).

There were some doubts about the effectiveness of the method of inducing reproduction and about the subsequent survival of the eggs and larvae of *H. bovis* in a locality that was so far south of the insect's established range (Scharff 1950). Consequently, these experiments were designed to determine whether the flies would behave normally in the mating and oviposition procedures and to assess the relationship between the number of eggs laid and the number of larvae that survived to cut holes in the backs of the cattle. Our observations also permitted some conclusions about the limitations of the spread of *H. bovis* southward.

METHODS.—Mature larvae and puparia were collected from South Dakota cattle brought into Texas. They were from the untreated checks of a previous experiment (McGregor & Bushland 1957). Some were caught in screen cones fastened over the warbles but most of them were collected from the concrete floor of screened pens in which the cattle were confined. The puparia of the two

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species were reared separately in jars containing loosely packed, slightly moist sand about 3 to 4 inches deep. They were maintained at 68° F. over most of the puparial period but were later transferred to 75° F. to hasten emergence. Only *H. bovis* adults were used because most of the *H. lineatum* adults had emerged before this experiment was begun.

The flies were not used until they had voided meconium and hardened to flight condition. Both the mating and oviposition procedures utilized tethered flight (Weintraub 1961): a male and a female, flying on threads fastened with paraffin wax to the mesonotum, were permitted to make contact in flight and induced to mate; then the tethered, fertilized female was induced to oviposit by forcing it alternately to fly and settle on a test calf. Flies that had to be stored for a short time until their turn were placed in shaded grass to avoid undue activity. Surplus flies were stored in individual containers in a refrigerator until they were needed on subsequent days. Preliminary trials were conducted with 13 pairs of flies to determine the weather conditions, times and locations for optimal mating and infestation of calves.

Month-old calves were infested between April 24 and May 24 with four replicates of 50, 100, 200, 500 and 1,000 eggs per calf (table 1). Each calf was infested with its entire complement of eggs at a single session, which was completed before the day was over. No attempt was made to restrict the infestation of any calf to the eggs of a single fly; this would have been impossible in any case for the calves infested with 500 or 1,000 eggs.

Samples of eggs from the first few mated females were incubated at about 95° F. to check their fertility. No observations were made on the infested calves for hatching of the eggs or invasion by the larvae. The calves were not protected from attack by wild flies because the experimental infestations were out of phase with the natural developmental cycle of *H. lineatum* (Bishopp *et al.* 1949) and because there was no danger of infestation by wild *H. bovis*.

The infested calves were checked periodically until grubs were detected in their backs, then were examined systematically every 2 to 3 weeks until the end of the season. Individual grubs were charted on maps of the animals' backs to obtain a cumulative total of the number of grubs that survived to cut holes. At the same time counts were made of the numbers of grubs present simultaneously in the backs to show the build-up and decline of the population. When emergence of the grubs was imminent the cattle were transferred to the screened, concrete-floored pens in which the mature grubs were collected. The grubs were identified to species at the time of dropping, although some were squeezed out and identified earlier as an interim check (table 1).

RESULTS AND DISCUSSION.—The rearing conditions of

Table 1.—Controlled infestation of Texas calves with *Hypoderma bovis*. Kerrville, Texas. 1956–57.

No. Eggs/Animal	Date of Oviposition 1956	Number of Grubs in Back, 1957							Total Grubs That Appeared in the Back	Survival (%)
		1/9	1/29	2/12	2/26	3/14	3/27	4/23		
50	4/26	1	4	9	9	7	4	0	13	26
	4/26	7	7	8	7	3	1	0	9	18
	5/ 9	14	14	12	10	3	0	0	18	36
	5/ 9	6	11	16	16	15	13	0	18	36
									Av. 14.5	29.0
100	4/24	20	21	21	23	2	1	0	26	26
	4/24	17	14[a]	11	9	1	0	0	18	18
	4/28	9	20	21	22	8	3	0	22	22
	4/29	8	17	17	22	17	1	0	22	22
									Av. 22.0	22.0
200	4/30	32	35	35	33[b]	—	—	0	40	20
	5/ 7	21	20	20	11	0	0	0	23	12
	5/ 8	0	0	0	0	0	0	0	0	0
	5/ 9	9	9	11	9	2	2	0	11	6
									Av. 18.5	9.2
500	5/ 9	27	33	34	32	4	1	0	36	7.2
	5/10	10	15	14	12	3	0	0	16	3.2
	5/10	36	66	67[c]	—	—	—	—	67	13.0
	5/14	1	1	1[d]	0	0	0	0	2	0.4
									Av. 30.2	6.0
1,000	5/17	1[d]	0	0	0	0	0	0	1	0.1
	5/23	2	2	2	1	0	0	0	3	0.3
	5/24	4	17	19	18	16	12	0	23	2.3
									Av. 9.0	0.9
Average of all tests		11.9	16.1	16.7	12.3	4.3	2.0	0		

[a] Not including four larvae removed 1/15/57.
[b] This animal placed in screened pen 2/12/57. No new larvae appeared after that date and the last larva dropped 4/3/57.
[c] This animal placed in screened pen 2/12/57. No new larvae appeared after that date. First larva dropped out 2/22/57 and last 4/6/57.
[d] None of these grubs completed their development.

H. bovis puparia were favorable for a survival of 85%. The pupation period was about 25 to 30 days. The flies that emerged were robust, vigorous and comparable in their actions to those observed under similar conditions in Alberta. The adult emergence of each day was about equally distributed throughout the 24 hours, although larger numbers were collected at 8 a.m. since this collection represented a 12-hour interval of emergence. Storage of adults up to 3 days in the refrigerator did not impair their vigor or interfere with the efficiency of the mating procedure.

During the preliminary mating trials we held groups of adults in containers under conditions favoring activity. In none of these instances did mating activity occur. Furthermore, unsuccessful attempts had been made previously at Kerrville to duplicate the results of Bishopp *et al.* (1926) in obtaining spontaneous mating of *H. bovis* in cages.

In mating trials by tethered flight in sunny weather, five vigorously flying pairs mated readily; four pairs did not mate but were poor fliers which stopped flying altogether when the temperatures rose above 90° F. in the afternoon; the remaining pair also did not mate though flying vigorously, but this pair was tested mainly at high temperatures in the afternoon. In rainy and overcast weather three pairs could not be induced to mate, but they mated readily the following day in clear sunny weather. All but one of the matings occurred in the morning at temperatures below 85° F.; the remaining one took place in the early afternoon at 88° F. All of the matings took place in the shade of buildings or trees. Consummation of the mating act thus seemed to depend upon vigorous flight and was subject to the same environmental factors that limited flight, namely rainy weather at one extreme and air temperatures above 85° to 88° F. at the other. Although these tests were not exhaustive, they provided sufficient guidance for the subsequent routine matings which accorded with the results of the preliminary tests. It was found that mating could be induced in the evening as well as the morning, thus confirming the conclusion that the main limiting environmental factor at Kerrville was high temperature.

In the mating trials the flies behaved similarly to those previously tested in Alberta (Weintraub 1961) except for one marked difference. On a number of occasions copulation did not take place though the flies made contact in flight and grasped each other in the proper alignment. In these cases it was necessary to permit the pair to rest in the shade for about 5 to 10 minutes, after which they copulated on the first or second contact in flight.

Oviposition behavior of the mated females also was similar to that previously observed in Alberta: *e.g.*, when a female was forced alternately to fly and settle on a calf it deposited a single egg almost every time it touched the calf. However, the females also could be induced to deposit a number of eggs on different hairs at one sitting by jerking the thread to tilt the fly, whereupon an egg would be laid with each tilt of the fly. This greatly facilitated infesting the calves with their complements of eggs and the time required was reduced by about one-half, so that the flies laid about two eggs per second. The limiting conditions for oviposition were the same as those for mating, *i.e.*, the flies could not be induced to oviposit in rainy or overcast weather nor in the extreme heat of the day (above 90° F.). They showed signs of heat stupor when exposed to the high afternoon temperatures. Consequently the routine infestations were also scheduled mainly for the mornings and early evenings.

The high constant temperature of 95° F. was not conducive to hatching in the samples of eggs incubated to test the success of the mating. However, all of the batches of eggs showed development of larvae to the stage where segmentally arranged spines were visible. This was considered sufficient evidence of fertilization and no further check was made of eggs in most of the infestations.

The surviving grubs began to cut holes in the backs in early January and achieved their peak numbers in mid-February (table 1). The peaks occurred about 2 months earlier than those of the native South Dakota populations (Lofgren *et al.* 1954). This discrepency resulted from the fact that under the controlled rearing conditions at Kerrville the adults emerged, and the cattle were infested, about 2 months earlier than the reported period of adult activity in South Dakota. There were no detailed correlations between the dates of infestation and the dates of either the first appearance or the peak numbers of grubs in the backs. In any one animal, grubs kept arriving in the backs over a period of about 2 to 3 months despite the fact that all of the eggs had been deposited within 1 day. This can only be accounted for by a more variable migration of the grubs within the host than has generally been considered.

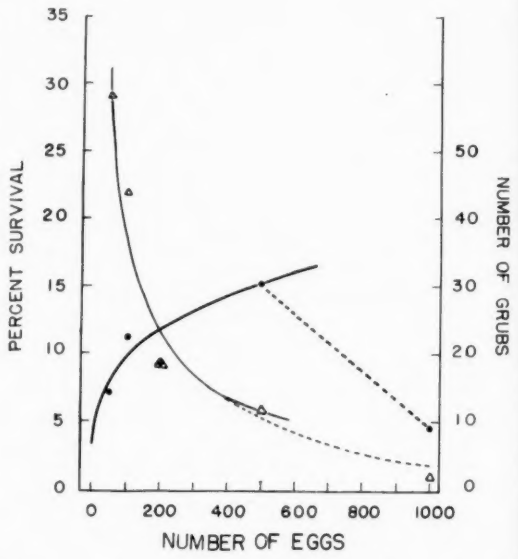

FIG. 1.—Number and per cent survival of grubs in calves infested with various numbers of eggs of *H. bovis* at Kerrville, Texas.

(●) = mean number of grubs ($Y = 5.07X^{.280}$).

(△) = mean per cent survival.

Solid lines were fitted by method of least squares, broken line by eye.

The relationship between the number of eggs and the number of surviving grubs fitted the power law below the level of 500 eggs, and took the particular form $Y=5.07X^{.280}$ (figure 1). However, the point for 1,000 eggs deviated strongly from the fitted curve. If that point is ignored the lines for both number and per cent survival are similar to those for a large series of infestations in Alberta in which 25 to 930 eggs/animal were used (Weintraub & Robertson, unpublished data). The validity of the 1,000-egg point is doubtful because the original infestations were not checked for viability. Many of the eggs might well have been infertile, or if fertile, might have succumbed to higher temperatures, for these infestations were conducted later in the season than those at the lower levels (table 1).

The pattern of survival (fig. 1) suggests that a density-related mechanism was in operation. We did not investigate the nature of this mechanism, but it seems to involve quantitative immune responses to single infestations similar to those reported to be acquired by older cattle through repeated annual infestations (Bishopp *et al.* 1926, Bevan & Edwards 1951, Scharff 1950). The power law curve for the number of surviving grubs indicates that the mortality factors increased proportionately throughout the range investigated. The descending broken line between the 500- and 1,000-egg levels would have indicated that the mortality factors intensified in that range were the line not based upon the doubtful 1,000-egg point. More infestations at the higher densities are required to fix the exact formula for the survival curve.

It is noteworthy that less variation was found below 200 eggs, the least occurring in the 100-egg infestations (table 1). This may be used as a guide for infesting cattle for the purpose of screening insecticides, in which replicates with uniform numbers of grubs are desirable.

CONCLUSIONS.—The adults of *H. bovis* may be induced to reproduce under Texas conditions and the resulting infestations are viable, but reproduction is curtailed severely by the high afternoon temperatures. However, the flies may avoid these high temperatures by mating and ovipositing in the morning (Bishopp *et al.* 1926). Therefore this limitation does not seem to be sufficiently severe to exclude the species from the South, although it could well slow the spread and prevent the establishment of heavy infestations. The recent spread of *H. bovis* into southern California (Scharff 1950) and Oklahoma (U. S. Dept. Agric. 1959) would suggest that no additional deterring factors operated in those areas. Probably a more important limiting factor further south is the high mortality of the puparia because of excessively high temperatures (Bishopp *et al.* 1926). Since *H. bovis* is a more serious pest of cattle than is *H. lineatum* it would be worthwhile to test this supposition by investigating the adaptive survival of the puparia under natural conditions.

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Toxicity of TDE to Different Instars and Strains of the Red-Banded Leaf Roller and the Response of a TDE-Resistant Strain to Parathion and Endrin[1]

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Abstract

The decline in control of the red-banded leaf roller, *Argyrotaenia velutinana* (Walker), with TDE over the past few years indicates the development of resistance to this insecticide in certain populations of leaf rollers. The possibility of such resistance in a Virginia strain was verified in the present study when fifth-instar larvae of a Virginia strain were found to be 28.4 times more resistant to TDE than fifth-instar larvae of a Canadian susceptible strain. The toxicity studies consisted of confining the larvae under a treated glass or plastic cup inverted on treated apple, and apple foliage. First-, second- and fifth-instar larvae of the TDE-resistant strain were investigated as to their response to TDE. It was found that LC_{50} second-instar larvae were 185.1 times more resistant to TDE than first-instar. The results showed also that second- and fifth-instar larvae had comparatively the same response to TDE at LC_{50}.

The toxicity of parathion and endrin to second-instar larvae of the TDE-resistant strain was investigated. It was concluded that parathion resistance is probably present, and that the TDE-resistant strain is susceptible to endrin.

Following the introduction of DDT to control the codling moth (*Carpocapsa pomonella* (L.)) the red-banded leaf roller (*Argyrotaenia velutinana* (Walker)) became a major pest in apple orchards throughout Virginia. Harman (1948) postulated that leaf roller outbreaks following the use of DDT were caused by the low toxicity of DDT to this pest and its toxic action against parasites and predators. In laboratory tests with treated grape leaves, Taschenberg (1949) found that TDE was very effective against 16- to 23-day-old larvae. Field tests showed that parathion was excellent in control of first brood, and good against second brood, and that TDE gave excellent control of second-brood leaf rollers (Glass & Chapman 1949, Graham 1949). Because of these results, the insecticides TDE and parathion have been used for controlling the red-banded leaf roller in apple orchards. However, in recent years the effectiveness of these insecticides in some Virginia orchards has declined to such an extent that resistance to TDE and parathion is indicated. Glass (1957), using laboratory tests, showed TDE resistance in newly hatched larvae when he found that two suspected resistant strains were 8.1 and 9.9 times as tolerant to TDE as a normal strain.

The present study was undertaken to verify resistance to TDE in a Virginia strain of the red-banded leaf roller. In addition, the response to TDE shown by different instars of a TDE-resistant strain, and the response of this strain to parathion and endrin have been examined. The experimental tests were conducted at the Winchester Fruit Research Laboratory of the Virginia Agricultural Experiment Station.

Strains.—Larvae of the red-banded leaf roller were obtained from three orchards chosen upon the basis of the number of years TDE had been applied in the orchard. The Grim strain consisted of larvae from an orchard near Winchester, which was suspected of having resistance to TDE as this material had been used continuously from 1949 until the present. Larvae of the Green strain were collected from an orchard near Mt. Jackson, Virginia and it was regarded as a TDE-susceptible strain because the known history of TDE usage extended over only 4 years and no insecticide was applied to the orchard during the 1959 season. Larvae of the Canadian strain, obtained from an orchard located near Simcoe, Ontario through the kindness of Mr. A. Hikichi of the Entomology Substation at Simcoe, were also classified as a TDE-susceptible strain, since TDE had not been applied from 1955 to 1959; no information was obtainable on use of insecticides in this orchard previous to 1955. Because sufficient larvae could not be reared for the laboratory tests it was necessary to collect the desired instars from the appropriate apple orchards. Newly hatched larvae were obtained from laboratory and field-collected egg masses.

Toxicological Procedures.—Initially, topical applications of TDE were made to second generation, fifth-instar larvae. Larvae from the Grim and Green strains were treated with droplets of technical TDE in acetone, employing a Dutky-Fest microapplicator. With this topical application the highest concentration feasible was 6.96 milligrams per gram of body weight. This concentration was equivalent to 133 pounds of 50% TDE per hundred gallons. However, at this concentration the highest mortality for these two strains was slightly more than 50% and the data obtained could not be adequately analyzed for graphical representation. Although this technique was abandoned, due to its failure to produce sufficiently high mortality, the technique did indicate that the Grim and Green strains show comparatively the same response to TDE and that TDE resistance is evident in both strains.

A different technique had to be devised whereby comparisons of susceptibility to TDE of larvae in any instar could be made. The technique used consisted of confining the larvae on young apples and apple foliage under glass or plastic cups, all surfaces exposed to the larvae having been dipped into the desired concentration prepared by water extension from 50% TDE wettable powder. With the aid of a camel's-hair brush, larvae were placed inside the treated cups and the cups inverted over the treated apple and apple foliage. A rubber band was doubled around the apple and cup to secure the cup to

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the apple, and low-melting paraffin was placed around the cup to prevent escape of the larvae. The larvae were exposed to the toxicant at a temperature ranging between 78° to 80° F. for a period of 48 or 96 hours, depending upon the larval stage treated. This enclosed larvae technique was employed in obtaining all of the data reported here.

The data obtained were plotted on logarithmic probability paper and regression lines were fitted to the points by the method outlined by Bliss (1935). When necessary, corrections were made for natural mortalities (Abbott 1925). A comparison of the LC_{50} values of the different strains and instars was used to determine the presence of resistance to TDE, and the response to parathion and endrin.

RESULTS.—A total of 565 third-generation, newly hatched larvae from the Grim strain and 403 newly hatched larvae from the Green strain were exposed to different concentrations of TDE for a period of 48 hours. The regression lines for the two strains are shown in figure 1. These results for first-instar larvae show that the Grim and Green strains have comparatively the same response to TDE. A small amount of the toxicant was necessary to give 50% mortality for first-instar larvae. This concentration for Grim strain was 0.14 and for the Green strain 0.09 gram actual TDE per 1,000 ml. The 0.14 gram value is equivalent to 0.23 pounds of 50% TDE per 100 gallons.

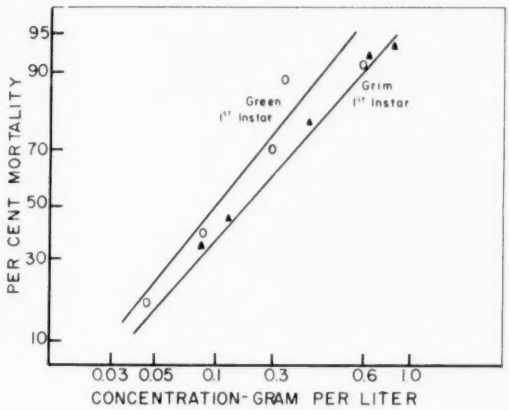

FIG. 1.—Toxicity of TDE to first-instar red-banded leaf roller larvae from the Grim and Green strains; mortality counts taken after 48 hours of continuous exposure to TDE by the enclosed larvae method.

Third-generation, second-instar larvae from the Grim strain only were exposed to different concentrations of TDE. A total of 270 larvae were treated during this series. At the highest concentration a sufficiently high mortality was not obtained after 48 hours of exposure; therefore, the exposure period was extended to 96 hours in order to obtain high mortalities. The regression line obtained is shown in figure 2, and the LC_{50} value was 25.5 grams actual TDE per 1,000 ml. This concentration is equivalent to 42.56 pounds of 50% TDE per 100 gallons, which is 185.1 times the amount of toxicant necessary to give an LC_{50} value for first-instar larvae. Third generation, fifth-instar larvae from the Grim strain and second generation, fifth-instar larvae from the Canadian strain were similarly treated for a period of 96 hours. A total of 335 larvae were treated from the Grim strain and 295 from the Canadian strain. The positions of the regression lines for fifth-instar larvae of the Grim and Canadian strains (figure 2) show a significant difference in susceptibility of the two strains to TDE. The LC_{50} value for fifth-instar larvae of the Grim strain was 25.0 grams actual TDE per 1,000 ml. of water, and that for the Canadian strain was 0.88 gram actual TDE per 1,000 ml. of water. Based on 50% kill, the Grim strain was 28.4 times as resistant to TDE as the Canadian strain. This would mean that one-half of the fifth-instar larvae of the population could tolerate 41.7 pounds of 50% TDE per 100 gallons. This concentration is 181.3 times the amount of toxicant necessary to give equal kill of first-instar larvae.

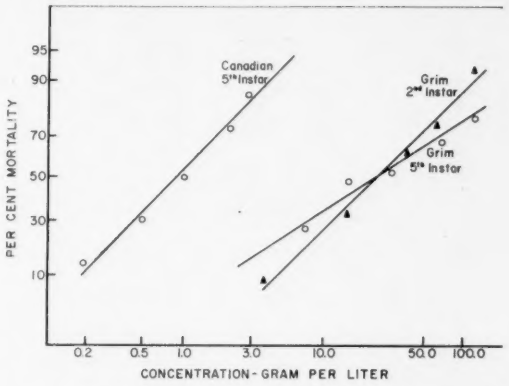

FIG. 2.—Toxicity of TDE to second- and fifth-instar red-banded leaf roller larvae of the Grim strain and fifth-instar larvae of the Canadian strain; mortality counts taken after 96 hours of continuous exposure to TDE by the enclosed larvae method.

Third-generation, second-instar larvae from the TDE-resistant Grim strain were exposed to parathion and endrin at different concentrations in order to obtain the dosage-mortality relationships for these two insecticides. Larvae were exposed to parathion for a period of 72 hours, and to endrin for 48 hours. Parathion had been applied in this orchard for approximately 10 years, whereas endrin had never been applied except as a ground spray to control mice. A total of 255 larvae were treated with parathion and 275 with endrin. Results for the parathion test (fig. 3) indicate that the LC_{50} value was 0.88 gram actual parathion per 1,000 ml. of water. This concentration is equivalent to 4.96 pounds of 15% parathion wettable powder per 100 gallons. Such a concentration necessary to give 50% kill would probably indicate a moderate degree of resistance to parathion. No valid inference can be made as to the magnitude of the apparent resistance to parathion in this strain as comparable data for a susceptible strain were not available.

The results from the endrin tests (fig. 3) indicated that

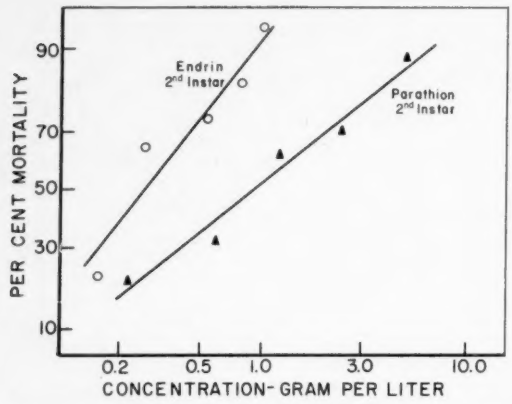

FIG. 3.—Toxicity of parathion and endrin to second-instar red-banded leaf roller larvae from the Grim strain; mortality counts taken after 72 hours (parathion) and 48 hours (endrin) of continuous exposure by the enclosed larvae method.

the TDE-resistant Grim strain was apparently susceptible to this insecticide. The LC_{50} value was equal to 0.25 gram actual endrin per 1,000 ml. of water. This concentration is equivalent to 0.25 pounds of 75% endrin wettable powder per 100 gallons.

DISCUSSION.—The unfed, newly hatched larvae from Grim and Green strains showed little difference in tolerance to TDE, and it was found that only a small amount of the toxicant was necessary to give 50% mortality of this instar. Second- and fifth-instar larvae of the Grim strain had approximately the same response to TDE. This is important from a practical standpoint since it is desirable to know which larval stage is susceptible to a particular insecticide. The method did not give true quantitative relationships of the toxicant for the two instars, as the amount of toxicant per gram of body weight of insect was not obtainable. However, the method did show that the LC_{50} values of the two instars was equivalent to approximately 42.0 pounds of 50% TDE per 100 gallons. Thus, from a practical standpoint, TDE would be eliminated for control of TDE-resistant larvae beyond the first instar. Fifth-instar larvae from the Grim and Canadian strains showed a response difference to TDE. Comparing the LC_{50} values of the two strains, it was found that the Grim strain required 28.4 times more toxicant than the Canadian strain. This verified resistance in the Grim strain, and indicated that TDE could not satisfactorily be used for control of the leaf roller in this orchard.

The results obtained from the parathion and endrin tests appear to eliminate parathion applications for controlling larvae in the TDE-resistant strain tested in this study. This is due to the relatively high concentration of parathion necessary to produce the desired mortality of second-instar larvae. However, second-instar larvae of the TDE-resistant strain were suceptible to concentrations of endrin which are acceptable for commercial use. Thus, the results obtained for TDE, parathion, and endrin in controlling larvae of a TDE-resistant strain of the red-banded leaf roller showed that of these only endrin may be effectively used.

The moderately high resistance to TDE shown by the leaf roller population in the Grim orchard may be due to several factors. Perhaps the main cause was the number of years chlorinated hydrocarbons had acted as a selective agent. DDT had been applied to this orchard since 1946 and TDE since 1948, thus approximately 30 to 40 generations of the red-banded leaf roller had been treated with chlorinated hydrocarbon insecticides in commercial spray operations. Red-banded leaf rollers subjected to selective pressure over such a period of time could be expected to develop moderately high resistance to the chlorinated hydrocarbon insecticides.

Since multiresistance to insecticides has been demonstrated in other insects it may be expected to occurr in the red-banded leaf roller. Insecticides from three different groups (Metcalf 1955) were studied during the course of this work; these were TDE (group I), endrin (group II), and parathion (group IV). The results of this study indicate that resistance had developed in a population of the red-banded leaf roller to TDE and parathion but not to endrin. The susceptibility to endrin could be expected as this material had not been employed for insect control in the orchards from which the larvae were collected. As a rule, the development of resistance in an insect population to insecticides of group I induces very little resistance to insecticides in group II or to organophosphate insecticides (Brown 1958). However, in most cases organophosphate pressure on an insect population does induce resistance to the two chlorinated hydrocarbon types as well as to the organophosphates (Brown 1958, Forgash & Hansens 1959). Organophosphates had been applied in the Grim orchard for approximately 10 years; during this period from 15 to 20 generations of selective pressure with organophosphates likely occurred. Thus, selective pressure of organophosphates over a period of 10 years, or 15 to 20 generations, would further support the indication of parathion resistance in the Grim orchard. Although resistance to endrin (group II) was not found in this study, it is possible that continued selection pressure by the organophosphates will produce cross resistance to this material. It may be expected that continued use of any group II insecticide on the Grim strain will eventually develop resistance to this group.

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The Ticks (Argasidae and Ixodidae) of Turkey[1]

DALE W. PARRISH[2]

ABSTRACT

Based on data gathered over a 13-month period, beginning in 1958, 19 species, representing 8 genera of ticks, are listed as occurring in Turkey. These include 5 *Haemaphysalis* species, 5 *Hyalomma*, 2 *Argus*, 2 *Boophilis*, 2 *Rhipicephalus*, 1 *Dermacentor*, 1 *Ixodes*, and 1 *Ornithodoros*. The physical features of Turkey are briefly described and a map is presented showing the four topographical areas into which the country may be divided. Areas in which each tick species occurs are shown opposite the species listed. Six species have been incriminated in the transmission of animal diseases in Turkey.

The Entomology Service, Fourth Epidemiological Flight, United States Air Force, conducted a preliminary survey of arthropods of public health significance occurring in Turkey, commencing in April 1958. This survey was conducted over a period of 13 months. Arthropods collected in the survey included ticks, mosquitoes, sand flies, deer flies, horse flies, and stable flies. In this paper, information is presented on the species of ticks collected in Turkey and their geographical distribution and hosts.

METHODS.—Collections were made from domestic animals and fowl in each of the four topographical areas of Turkey. Cattle, sheep, goats, water buffaloes, donkeys, horses, camels, dogs, cats, and domestic fowl were examined. Regional veterinary personnel, Turkish Ministry of Agriculture, arranged to have cattle, sheep, goats, and water buffaloes brought into villages from the open range for examination. Whenever camel trains were observed along the roads they were stopped for examination for ectoparasites. Donkeys, horses, dogs, cats, and domestic fowl were examined in the villages. Specimens were carefully removed from the host and preserved in 23-ml. vials of 70% alcohol. Specimens were also collected by dragging vegetation with a flannel cloth.

DISTRIBUTION AND HOSTS.—The hosts from which ticks were collected included man, domestic animals and fowl, and vegetation by flannel drag. The species of ticks collected between April 1, 1958 and June 1, 1959 in the provinces of Izmir, Diyarbakir, Elazig, Seyhan, Antalya, Bursa, and Samsun are listed in table 1. Eight genera and 19 species of ticks were identified from field collections made in the seven provinces of turkey covered in this survey.

Turkey is situated between 36° and 42° north latitude, 26° and 55° east longitude. It is bounded by the Black Sea on the north, the Russian Caucasus on the northeast, Iran on the east, Iraq, Syria, and the Mediterranean Sea on the south, Greece and the Aegean Sea on the west, and Bulgaria on the northwest. There are 4,429 miles of coastline, deeply indented and fringed with islands along the Aegean Sea. The area of Turkey is 296,503 square miles, 96.88% of which lies in Asia and the remaining 3.12% in Europe. Turkey-in-Europe is divided from Turkey-in-Asia by the Dardanelles, the sea of Marmara and the Bosphorus. The European portion of Turkey is about the size of New Hampshire, while the Asiatic portion is somewhat larger than the combined areas of Pensylvania, Virginia, West Virginia, Ohio, Indiana, and Illinois.

Topographically, Turkey is divided into four areas, namely, the Coastal Plain, Anatolia, Eastern Anatolia, and Southeast Anatolia. Tick collections were made from domestic animals and fowl in one or more provinces located in each topographical area (fig. 1). It is anticipated that additional surveys will be made in the future

Table 1.—Species of ticks collected in Turkey, their distribution and hosts.

SPECIES	REGION	HOST OR SOURCE
ARGASIDAE Canestrini		
Argus persicus (Oken)	I-II-III-IV	Domestic fowl
Argus reflexus (Fabricuius)	I-II	Man, vegetation
Ornithodoros lahorensis (Neumann)	I-II-III-IV	Man, cattle, sheep, camel, vegetation
IXODIDAE Murray		
Boophilus annulatus (Say)	I	Cattle
Boophilus calcaratus (Birula)	I-II-III	Cattle, horse, donkey, water buffalo, sheep, vegetation
Dermacentor marginatus (Sulzer)	I-II-III	Cattle, sheep, goat
Haemaphysalis concinna Koch	I-II-III	Cattle, water buffalo
Haemaphysalis inermis Birula	I-II-III	Horse, sheep
Haemaphysalis otophila Schulze	I-II-III	Cattle, dog, cat
Haemaphysalis punctata (Canestrini and Fanzage)	I-II-III-IV	Cattle, horse, goat, camel
Haemaphysalis sulcata (Canestrini and Fanzage)	I-II	Cattle, goat
Hyalomma anatolicum Koch	I	Cattle
Hyalomma detritum Schulze	I-II-IV	Cattle, horse, donkey, sheep, goat
Hyalomma dromedarii Koch	IV	Camel
Hyalomma excavatum Koch	I-II-III-IV	Cattle, horse, water buffalo, goat, camel
Hyalomma savignyi (Geruais)	I-II-III-IV	Cattle, water buffalo, camel, dog
Ixodes ricinus (Linnaeus)	I-II-III-IV	Cattle, water buffalo, sheep, dog, vegetation
Rhipicephalus bursa (Canestrini and Fanzage)	I-II-III-IV	Cattle, horse, sheep, goat, camel, dog
Rhipicephalus sanguineus (Latreille)	I-II-III-IV	Cattle, horse, water buffalo, sheep, dog

[1] Accepted for publication September 29, 1960. Partial cost of publication of this paper was met by the author.

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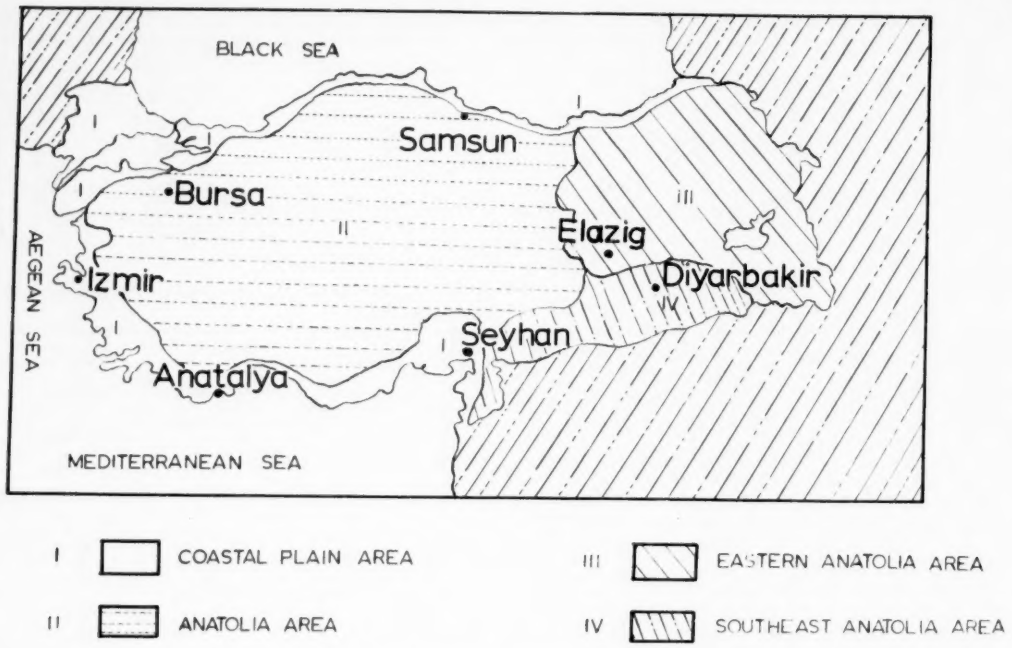

FIG. 1.—Map of Turkey showing the four topographical areas.

to include all the provinces of Turkey, and further, will include the examination of live specimens for isolation of disease producing agents.

The importance of ticks as transmitters of disease agents to man and animals in Turkey has not been fully explored. This is especially true with respect to the transmission of human diseases. The species of ticks which have been incriminated in the transmission of animal diseases are listed in table 2.

Table 2.—Disease agents transmitted to animals by ticks in Turkey.[a]

TICK SPECIES	SPECIFIC AGENT
Rhipicephalus bursa	*Piroplasma ovis*
Haemaphysalis otophila	*Babesiella ovis*
Boophilus calcaratus	*Piroplasma bigemina*
Hyalomma detritum	*Theileria annulata*
Argus persicus	*Spirochaeta gallinarum*
Ornithodoros lahorensis	*Coxiella burneti*

[a] Personal communication, Dr. Hasib Kurtpinar, Head, Laboratory of Parasitology, Veterinary Bacteriology and Serology Institute, Ankara, Turkey.

COMMON NAMES OF INSECTS

Approved by the Entomological Society of America

By Jean L. Laffoon, Chairman, Committee on Common Names of Insects

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Studies of an Integrated Control System for Hornworms on Tobacco[1]

F. R. Lawson, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*, R. L. Rabb, F. E. Guthrie, and T. G. Bowery, *North Carolina State College, Raleigh*[2]

Abstract

Hornworms on tobacco, *Protoparce sexta* (Johan.) and *P. quinquemaculata* (Haw.), are now controlled with insecticides. When *Polistes* wasps were induced to nest in shelters erected around the fields or moved from the nesting sites to the field, populations of fifth-instar hornworms were reduced by about 60% and damage by 74%. However, the predators had little effect on tobacco budworms (*Heliothis virescens* (F.)) and did not prevent severe damage when hornworm populations were very high and wild populations of wasps were low. When TDE or endrin was applied to the top four or five leaves of the plant when the first larvae reached the fourth instar, control was obtained with about one-fifth the recommended dosage. A system of integrated biological and insecticidal control adjusted to meet local conditions is suggested.

The experiments reported here were part of a general investigation of the biology and control of the hornworms *Protoparce sexta* (Johan.) and *P. quinquemaculata* (Haw.) that was conducted in North Carolina in 1952–56. Although both species of hornworms occur in North Carolina, *sexta* is the more abundant. They have similar life histories and, with minor exceptions, are attacked by the same enemies (Lawson 1959).

Recommendations for hornworm control vary with the location. Most States recommend that tobacco stalks be cut soon after harvest to reduce the next year's population (Caffrey 1956). Gilmore (1938) suggested fall plowing to destroy pupae; hand picking is sometimes suggested, but the main reliance is placed on insecticides, usually TDE or endrin in amounts varying with the size of the plants and the method of application.

Control of hornworms with insecticides is not completely satisfactory. Although the residues of recommended materials when applied at the usual dosage have no appreciable effect on the flavor or aroma of the finished product, tobacco manufacturers would prefer to have no toxic materials added to the natural leaf. For that reason it would be desirable to eliminate residues on the crop or at least reduce them below present levels. Moreover, there is the usual objective of reducing costs. An attempt was made to solve these problems by the use of biological control agents and certain modifications of the standard chemical control practices.

Biological Control.—*Parasites.*—Although hornworms are attacked by several parasites, Lawson (1959) found that at Oxford, North Carolina only the braconid *Apanteles congregatus* (Say) killed more than 1% of the population on the average. This species killed less than 1% of the first brood, 9% of the second, and 23% of the third. In the fall of 1952 large numbers of cocoons of this parasite were collected and placed in a refrigerator. The following spring they were released in a small isolated field of tobacco where there was a fairly large population of eggs and young hornworms. Nearly all the hornworms were killed by other enemies and no parasitized larvae were seen. Further experiments with a parasite so severely limited by other enemies did not appear promising.

Disease.—White (1923) obtained only about 12% infection by oral inoculation with *Bacillus sphingidis* White, the causative organism of hornworm septicemia. Lawson (1959), using an unknown pathogen, obtained a high mortality in the laboratory by this method, but limited field tests were not promising. Rabb *et al.* (1957) obtained a high mortality in field tests of *Bacillus thuringiensis* Berliner.

Predators.—Hornworms are attacked by several species of *Polistes* wasps. These predators kill 50% to 98% of the larvae present in tobacco fields, and hornworm outbreaks occur only when predation is low. Manipulation of the predator population as a means of biological control appeared possible when it was discovered that *Polistes* wasps would nest in artificial shelters that could be moved from place to place. Ballou (1913) reported, "In St. Vincent and St. Kitts, *Chalcis* sp. and the Jack Spaniard wasp (*Polistes annularis* (L.)) control the cotton worm to a noticeable extent, and planters are encouraging the wasps by erecting rough shelters for them in and near the cotton fields."

In 1955 an experiment was designed to test the use of wasp shelters in control of hornworms. This experiment was repeated in 1956 with some changes. The arrangements were randomized blocks. All plots were about half an acre in size and were near a grower's field. Each block contained two plots; one was an untreated check with natural populations of *Polistes*, and the other had wasp shelters placed on one or more sides and was called the protected plot. Both plots were located on the same farm, but at least 300 yards apart to keep the wasps from attacking hornworms in the check. Each block was replicated on another farm, at least 10 miles away. The plots were really separate fields.

In 1955, 18 wasp shelters (figs. 1 and 2) were placed on the borders of each protected plot. In addition, 184 shelters of the type shown in figure 3 were put in various habitats some distance from the plots to serve as a reserve supply of colonies. By replacing the screen with a short board to prevent the escape of adults, colonies in shelters of this type can be moved from one place to another. In 1956 the number of shelters around the protected plots was increased to 40 to 47 per plot, except at Clayton.

In 1955 the five localities included in the experiment extended from Virgilina on the northern border of North Carolina to Selma near the middle of the State. In 1956 there were three localities in Columbus County, near the South Carolina border, one at Clayton, and one at Oxford.

The shelters were placed around the plots in March before the wasps had begun building nests. The species

[1] Accepted for publication July 21, 1960.

[2] In cooperation with the North Carolina Department of Agriculture.

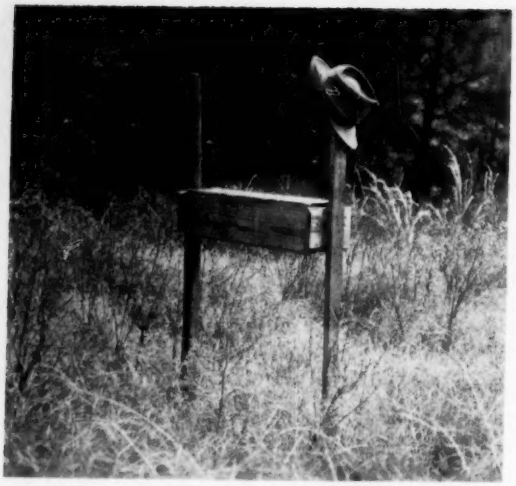

FIG. 1.—Wasp shelter.

FIG. 2.—Wasp shelter turned upside down to show method of screening.

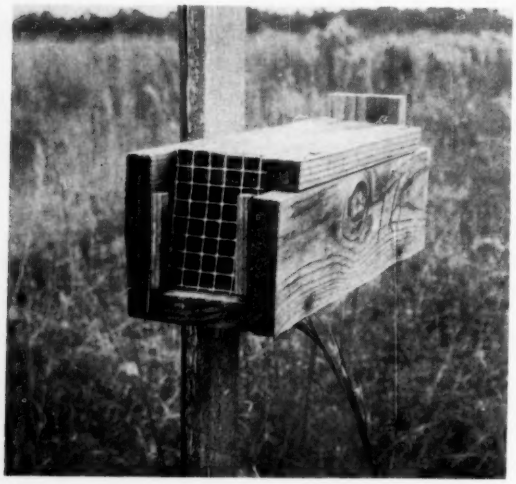

FIG. 3.—Movable wasp shelter.

nesting in the shelters and their relative abundance are indicated in table 1. These figures are based on 323 shelters in 1955 and 468 in 1956. The list includes all the species known to occur in the area (Rabb 1960). *P. exclamans* and *fuscatus* were far more numerous than the other species in both years and at all locations.

Table 1.—Species of *Polistes* nesting in artificial shelters in North Carolina.

SPECIES	NUMBER OF COLONIES PER 100 SHELTERS	
	1955	1956
exclamans Vier.	36.2	37.0
fuscatus (F.)	42.7	33.1
metricus Say[a]	0.3	4.7
hunteri Bequaert	.3	3.6
bellicosus Cresson	0	0.9
rubiginosus Lep.	1.6	.2
annularis (L.)	3.7	.2
Unidentified	3.4	7.1
Total	88.2	86.8

[a] *Metricus* may sometimes have been included with *fuscatus* owing to the difficulty in separating them in the field.

Many of the original nests were later abandoned. In 1955 an average of 20 nests were built per 18 shelters and 15 were still inhabited at the time hornworms appeared except at one plot where the original number was 15 and all but three were abandoned. To compensate for this loss, 29 colonies were transferred from another locality to this plot in June. Three of these became inactive within a week, and five more later in the season. After this experiment was completed shelters still occupied in August were moved again, together with nine additional ones. Only one colony was lost after this move. In 1956 many colonies were moved more than 100 miles. All of the species except *annularis* have been moved successfully.

In 1956 an average of 31 nests were built in 40 to 47 shelters at each plot. At the time hornworms appeared a mean of 24 colonies were still active. Only a few nests were started at the Clayton plot and more were moved in to make a total of 20 in June and 28 in July. This plot had additional colonies placed 400 to 800 feet from the tobacco.

The effect of protecting plots with wasp shelters is shown in table 2. The mean number of eggs laid was considerably higher in the plots with shelters than in the checks, but the difference was due to other factors, such as the condition and size of the plants.

Even though egg populations were higher in the protected plots, populations of fifth instars were consistently lower except for the second brood in 1956. The high mean in this brood was due almost entirely to a single station at Clayton, where the highest egg population recorded in 5 years of study produced a severe outbreak. When the means were adjusted for egg populations by covariance, the difference was significant and the control was about 60%.

Estimates of the number of leaves eaten were complicated by damage due to budworms (*Heliothis* spp.) in addition to hornworms, but show about the same trend as the population data. Again there was a reversal of the

Table 2.—Effect of wasp shelters on hornworm populations and damage.[a]

Year and Brood	No. of Wasp Colonies	No. of Hornworms on 25 Plants				No. of Leaves Eaten per 170 Plants[b]	
		Eggs		Fifth-Instar Larvae			
		Protected	Check	Protected	Check	Protected	Check
1955							
First	18.4	40.3	32.7	0.9	2.5	42.5	69.3
Second	14.6	15.3	11.1	1.4	2.3	11.7	25.2
1956							
First	22.8	14.8	7.0	0.7	1.4	16.6	24.0
Second	—	45.4	32.4	4.5	2.1	159.1	25.4
Mean	—	28.9	20.8	1.9	2.1	59.1	32.3
Adjusted mean	—	—	—	1.1[c]	2.8[c]	18.9	72.5

[a] All values are an average of five localities per year per brood, except for first-brood damage in 1955, which included only three.
[b] First-brood damage by both insects, second-brood mostly by hornworms.
[c] Difference significant at 1% level.

expected pattern in the second brood in 1956 because of the high population at Clayton. When the means were adjusted for egg populations by covariance, the difference was not significant although there was an indicated reduction in damage of 74%.

Average figures are not always the best indicators of effectiveness. A substantial reduction in populations or damage means very little if the population is still so high that the field must be treated with insecticides. In the 10 paired plots in these experiments there were two broods in each plot, making a total of 20 possible comparisons. In the checks there were 14 populations of fifth instars below the treatment threshold and six outbreaks. The only outbreak in the protected plot was at Clayton in 1956.

The budworm populations found in the plots were not greatly affected by *Polistes*. Although the wasps prey on budworms, much of the damage from these pests is done while they are within the buds and protected from predators (Rabb & Lawson 1957). In 1956 three localities in Columbus County had very heavy infestations of budworms. Two of these localities had less damage in the protected plot, but the third had more.

CONTROL WITH INSECTICIDES.—*Timing and Number of Applications.*—The number of insecticide applications made by growers to control hornworms may vary from one to four in the same vicinity in the same year. Some growers treat when the hornworms are small, others wait until fifth instars appear.

Tobacco requires about 90 days from the time the plants are set in the field until the harvest is completed. During this period there are ordinarily two distinct broods of hornworms. The eggs in each brood are laid in a period of 30 to 40 days. Lawson & Rabb (Unpublished data) found that about 80% of the tobacco consumed by a brood of hornworms in the field was eaten by fifth instars.

Since only the fifth instars cause appreciable loss, the first treatment against each brood can be delayed until these appear, but when bad weather forces postponement of the application there may be considerable loss. Thus it is prudent to make the application a few days earlier. Furthermore, preliminary experiments in 1955 indicated that earlier applications prevented some loss. Two ¼-acre plots in separate fields were sprayed with 0.2 lbs. of TDE per acre when the first fourth instars appeared. Hornworm damage amounted to 10 and 6 leaves per 170 plants. The remaining portions of the same fields were treated by the grower with TDE dust 4 and 5 days later. The amount of dust applied was not measured but probably exceeded a pound of TDE per acre. Damage in the dusted plots was 17 and 22 leaves.

Madden & Chamberlin (1945) found that the mean time required for the development of the egg and first three instars of *P. sexta* in the insectary in Florida was 14.2 days. At Oxford in a sample of 55 hornworms observed in the field the mean was almost the same, but 13% reached the fourth instar in 12 days. Thus, the first treatment should be made 12 to 14 days after the beginning of a brood.

If properly applied, this treatment will control all larvae present at the time of application and those hatching from eggs laid on the sprayed foliage. Very few hornworm eggs are laid on leaves less than 6 inches in length. Measurements of 10 tobacco plants in a typical stand in 1954 showed that new leaves reached this size in 6 to 8 days. Thus eggs may hatch and the larvae reach the fourth instar on untreated foliage in 18 to 20 days after the first treatment. However, these larvae may also be killed by residual insecticides. In the biological studies several hundred hornworms were located and examined daily from the time of hatching until they disappeared. The first and second instars moved about very little and were usually found on the leaf on which they hatched, but third and larger instars moved frequently from leaf to leaf. Table 3 shows the mortality of larvae hatched from eggs laid on new growth in fields treated with insecticides when the plants were growing rapidly. The mortality of the first two instars was similar in treated and untreated check fields, but in the treated fields third instar mortality was so high that very few hornworms reached the fourth instar and none the fifth.

Table 3.—Mortality of hornworm larvae hatching 13 to 17 days after treatment with insecticides in three fields at Oxford, N. C.[a]

Treatment	No. of Hornworms	Per Cent Mortality by Instars				
		1	2	3	4	5
TDE and parathion	19	42	21	37	0	0
Endrin	45	49	33	16	2	0
Untreated	32	41	29	9	12	9

[a] Larvae hatched June 16–20, 1953.

Guthrie *et al.* (1959) gave data showing that in a heavy and continuous infestation there were many fourth and fifth instars in the check 3 weeks after treatment, but very few in plots sprayed with TDE and endrin. As a result of such delayed mortality and additional losses of hornworms to natural enemies, one application per brood is all that is needed in many fields. For example, at Greenville, North Carolina in 1954, one application of TDE at the rate of 0.2 pound per acre was made when the first fourth instars appeared in a field that had a high population of second-brood hornworms. Damage in the untreated half

of the field was 295 leaves per 170 plants, in the sprayed half it was 14 leaves, a reduction of 95%. Another severe outbreak at Clayton in 1956 was also controlled satisfactorily with one application.

However, in many cases the determination of timing and number of applications needed for hornworm control is complicated by infestations of budworms which can be controlled by the same insecticides. Guthrie *et al.* in large scale tests, applied TDE or endrin when counts showed a population of five budworms of any size, or five third instar hornworms, per 50 plants. Although the average number of applications applied to these plots was less than the number applied by growers to adjacent fields, the combined loss from budworms and hornworms was about the same.

Control with Reduced Dosages of Insecticides.—Lawson (1959) found that natural enemies killed about 90% of the hornworms infesting tobacco before they reached the fifth instar. Gilmore (1938) and Madden & Chamberlin (1945) observed that a high percentage of the eggs were laid on the top four or five leaves of the plants. These facts suggested that control might be obtained by treating only the upper leaves. Hornworms on these leaves might be killed by the insecticide and those on the lower leaves carried away by predators or killed when they become large enough to wander about.

This idea was first tested in a half acre plot at Greenville in 1954. Hornworm damage was reduced by 95%. In 1955 and 1956 the top spray method was tested on the replicated plots used in the *Polistes* experiments previously described. About one-fourth of each half acre plot was sprayed. The insecticides were directed to the upper four or five leaves of the plants, so that the lower leaves received only the material that was not intercepted by the upper leaves. No counts were made of populations in the sprayed plots except that fifth instars were counted in 1956. Damage was estimated in all plots and in adjacent fields treated by growers. The results are given in table 4.

In 1955 no attempt was made to separate damage from budworms and hornworms. In 1956 there was a heavy outbreak of budworms, but very few hornworms, in Columbus County. However, there was no significant difference between insecticidal treatments in the amount of damage, but there were more fifth instars on the plots top-sprayed with TDE than on those receiving full coverage of endrin.

No distinction has been made in table 4 between plots protected by wasp shelters and unprotected plots. If the data are tabulated so that the comparison can be made there is a total of nine paired tests. In the plots treated with TDE top spray the mean loss to hornworms was 8.58 leaves per 170 plants in the unprotected plots as compared with 5.58 in the protected plots. The difference was significant. Thus the use of wasp shelters reduced damage by 120 leaves per acre in addition to the reduction caused by the spraying.

Guthrie *et al.* (1959) tested top sprays in growers' fields at 24 locations in 1957 and 21 in 1958. The method proved more effective than growers' treatments against hornworms in 1958, probably because of better timing, but otherwise there were no significant differences between this method and full coverage or growers' treatments. The average number of applications was 2.2 in the top spray plots and 3.5 in growers' fields. The residue of TDE on the cured leaves was reduced by 45% and that of endrin by 66%.

Table 4.—Reduced dosages of insecticides applies as top sprays compared with full coverage and growers' treatments for the control of budworms and hornworms.

Treatment	Pounds of Insecticide per Acre	Fifth-instar Hornworms per 25 Plants	Leaves Eaten per 170 Plants
Hornworms and budworms—all localities 1955 (8 replicates)			
Unsprayed	0	—	53.2
TDE top spray	0.2	—	21.4
Grower's (TDE)	?	—	14.1
L.S.D. at 5% level	—	—	14.2
Mostly budworm damage, Columbus County, 1956 (3 replicates)			
Unsprayed	0	—	240.3
TDE top spray	0.2	—	39.7
Endrin top spray	.04	—	32.8
Endrin full coverage	0.04–.15	—	18.3
Grower's (TDE)	1 –1.3	—	15.4
L.S.D. at 5% level[a]	—	—	137.5
All localities, hornworms, 1956[b] (8 replicates)			
Unsprayed	0	2.7	38.0
TDE top spray	0.2 –0.25	0.7	9.9
Endrin top spray	.04– .10	.4	9.4
Endrin full coverage	.04– .40	.1	5.3
L.S.D. at 5% level	—	1.4	17.5

[a] Grower's treatments omitted.
[b] Budworm damage at Clayton included.

Integrated Control.—From the experiments described it is apparent that no one method of control is entirely satisfactory. Recommendations for complete coverage with highly toxic insecticides are designed to control the most severe outbreaks in the shortest possible time. They are highly effective for this purpose, but there are few severe outbreaks and with care they can be controlled before damage occurs. Thus, farmers use a greater number of applications at a higher dosage than they really need.

Biological control with *Polistes* wasps is inexpensive and leaves no residue, but this method by istelf will not give adequate control of hornworms under all conditions nor will it prevent damage by budworms.

Small dosages of insecticides applied as top sprays give reliable and adequate control of both budworms and hornworms whether applied every 2 weeks on a preventive schedule or when counts indicate treatments are needed. The preventive treatments are more expensive and leave more residue (Guthrie *et al.* 1959).

A combination of biological and chemical control might reduce both costs and residues over any one method used alone. The treatments that leave the most residue are those applied against second-brood hornworms because the tobacco may be harvested soon after application (Bowery *et al.* 1959). In many cases the need for late applications could be prevented by the wasp shelters. This method would be most useful where severe outbreaks of budworms or hornworms are infrequent, but would prevent some loss even when insecticides are necessary.

Pathogens could be substituted for insecticides in suitable situations. The efficiency of any material would be greatly increased by proper timing, and by limiting treatments to those fields where damaging populations are indicated by actual counts. Full coverage of insecticides might sometimes be useful if treatments were delayed until after the hornworms had reached the fifth instar.

Such an integrated program would be more economical than the system now used and would reduce residues to a fraction of the present levels.

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Residues in Tissues and Eggs of Poultry Receiving Co-Ral (Bayer 21/199) in the Feed[1]

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ABSTRACT

Radiophosphorus Co-Ral® (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothioate or Bayer 21/199) was mixed in laying mash at 100 p.p.m. and fed to laying hens for a maximum of 7 days. The highest concentration of total radioactive residues was in the liver and kidney; deposition of Co-Ral or metabolites in the fat was minor. The liver, kidney, and gizzard contained the highest concentration of acetonitrile-soluble materials. Acetonitrile-soluble residues were not present in any tissue after the hens were returned to normal feed for 7 days. Small but detectable amounts of acetonitrile-soluble materials were present in egg yolks analyzed at 11 to 15 days after treatment, but none was present in the albumin.

About 79% of the radioactivity consumed in the feed was excreted in the feces by 28 days posttreatment; more than 85% of the excreted radioactive materials were hydrolytic products. Unchanged Co-Ral, the oxygen analog of Co-Ral(*O,O*-diethyl phosphoric acid) and *O,O*-diethyl phosphorothioic acid were isolated and characterized from the feces.

Co-Ral® (*O,O*-diethyl *O*-(3-chloro-4-methylumbelliferone) phosphorothioate or Bayer 21/199) appears promising for use on poultry, since it was effective against certain ectoparasites (Kraemer 1959) and the residues in tissues and eggs of laying hens dusted with the material were negligible (Dorough *et al.* 1961). Poor absorption through the skin and rapid degradation of the absorbed Co-Ral partially explained the low order of toxic residues in tissues and eggs of poultry (Dorough *et al.* 1961) and mammalian tissues (Krueger *et al.* 1959, Robbins *et al.* 1959, Vickery & Arthur 1960). Co-Ral administered orally to mammals was also rapidly degraded to diethyl phosphoric and diethyl phosphorothioic acids (Lindquist *et al.* 1958, Kaplanis *et al.* 1959), and the liver was largely responsible for the inactivation processes (O'Brien & Wolfe 1959). However, the possibility existed that oral intake of Co-Ral by poultry might result in higher residues in tissues and eggs than when dusted on the birds.

The deposition of Co-Ral in tissues and eggs, excretion rates, and the isolation and characterization of metabolites were investigated following administration of radiolabeled Co-Ral in the feed of laying hens.

PROCEDURE.—*Incorporation of Co-Ral in Laying Mash.*—Twenty pounds of laying mash was weighed and placed in a No. 3 galvanized tub. One gram of radiophosphorus Co-Ral (supplied by the Chemagro Corporation, specific activity 2 mc./gm., May 5, 1959) was dissolved in 500 ml. of acetone and sprayed over the feed. The feed was mixed until the P^{32} was evenly distributed as determined by counting (end window Geiger-Müller tube) several 100-mg. samples. The concentration of P^{32} Co-Ral in the feed was 100 p.p.m.

Feeding of Hens.—Twenty-one Rhode Island hens were housed singly in wire cages equipped with dropping boards. Egg production of the hens was 20% to 30% per day and the average weight of the hens was 2.734

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kg. The hens were provided radioactive feed and water free-choice. The radioactive feed was placed in pint cardboard cartons, which were fastened to the outside of the cages. Feed lost from the carton during feeding by the hens was collected on protecto-paper. Records were kept on feed consumption per hen per day (table 1). The hens were fed the Co-Ral-feed-additive for a maximum of 7 days and then certain hens were returned to normal feed.

Sacrificing Intervals.—One day after placing the hens on radioactive feed, 3 of the 21 hens were sacrificed for residue determinations in the tissues. Another 3 hens were sacrificed at 3 days and another 3 hens at 7 days after beginning the experiment. At the end of the 7-day feeding period, the remaining 12 hens were returned to normal feed. These were sacrificed in groups of three at 3, 7, 14, and 21 days after the return to normal feed.

Residue Determinations, Excretion Rates, and Metabolism Studies.—Tissues analyzed, determination of residues, and extraction procedures for tissues and eggs were as described for hens dusted with P^{32} Co-Ral (Dorough *et al.* 1961). Untreated hens were not used to determine the percentage recovery of Co-Ral added to tissues, since good recoveries were obtained from the tissues of white Leghorn hens used in the dust experiment (Dorough *et al.* 1961). Procedures used in the determination of total P^{32} materials in the droppings, in calculation of percentage hydrolysis, and for the isolation and characterization of Co-Ral metabolites in the feces by column partition and anion exchange chromatography have been described previously (Dorough *et al.* 1961).

RESULTS.—*Residues in Tissues.*—The p.p.m. Co-Ral equivalents in several tissues of hens at various intervals after beginning the experiment are given in table 2. During the 7-day period on radioactive feed, the highest concentration of total P^{32} residues was in the liver and kidney, and these residues declined sharply when the hens were returned to normal feed. Deposition of total residues in the fat was minor. Slightly more residues were noted in the muscle tissue of the drumstick than in the breast. Accumulation of total P^{32} materials in the bone was rapid and appeared to reach a maximum at 10 days after beginning the experiment. The skin contained only negligible residues and no P^{32} residues were recorded from the feathers (table 2).

Tissues containing greater than 0.02 p.p.m. total P^{32} residues were fractionated (Anderson *et al.* 1959) to determine the p.p.m. acetonitrile-soluble residues. The liver, kidney, and gizzard contained the highest concentration of acetonitrile-soluble radioactive materials throughout the 7-day feeding of radioactive feed, but these residues were soon dissipated when the hens were returned to normal feed (table 3). Toxic residues in other tissues were only slightly above the lower limit of sensitivity of the analytical method. No toxic residues were recorded in any tissue after the hens had been returned to normal feed for 7 days (table 3).

Residues in Eggs.—Considerably more total P^{32} residues accumulated in the yolk than in the white or shell of eggs (table 4). The highest concentration of total P^{32} materials appeared in egg yolks at 7 to 16 days after the hens were initially placed on radioactive feed. The amount of total P^{32} residues in all parts of the egg was somewhat variable, probably resulting from the different amounts of feed consumed by the hens (table 1). Egg production was low; consequently, the number of analyses was limited.

Small but detectable amounts of acetonitrile-soluble radioactive materials were present in the yolks of eggs analyzed at 6 to 8 days after the hens were returned to normal feed (table 4). The occurrence of these residues (table 4) did not coincide with the decline of residues

Table 1.—Average amount of feed consumed by hens fed Co-Ral mixed in the feed.[a]

Days After Treatment	No. of Hens	Av. Feed Consumed/Day/Hen (gm.)	Av. P^{32} Co-Ral/Kg. Body Weight[b] (mg.)
1	21	46.56	1.767
2	18	39.36	1.541
3	18	49.50	1.934
4	15	63.69	2.513
5	15	43.85	1.744
6	15	46.62	1.838
7	15	62.15	2.436

[a] Hens fed P^{32} Co-Ral in feed at 100 p.p.m. for a maximum of 7 days.
[b] Average weight of all hens (21) was 2.734 kg.

Table 2.—Total p.p.m. Co-Ral equivalents in tissues of laying hens fed radioactive Co-Ral mixed in the feed at 100 p.p.m.

	Days on Radioactive Feed—p.p.m.[a]			Days on Normal Feed—p.f.m.[a]			
Tissue	1	3	7	3	7	14	21
Blood	0.25	0.13	0.23	0.07	0.04	<0.02	<0.02
Bone	.13	.14	.32	.69	.55	.55	.65
Brain	.03	< .02	.12	.08	.09	.09	< .02
Breast	.07	.08	.05	.03	< .02	< .02	< .02
Drumstick	.04	.04	.17	.25	.13	.12	.10
Fat	.03	.04	.05	.04	< .02	< .02	< .02
Feathers	< .02	< .02	< .02	< .02	< .02	< .02	< .02
Gizzard	.07	.07	.20	.13	.12	.07	.10
Kidney	2.01	1.90	3.16	1.12	.67	.32	.09
Liver	2.60	1.97	4.05	1.07	.59	.20	< .02
Skin	.08	< .02	.17	< .02	.04	.03	.03

[a] Total Co-Ral equivalents before fractionation to determine toxic residue; averaged from three hens sacrificed at each time interval with duplicate analyses per tissue.

Table 3.—Acetonitrile-soluble radioactive materials in tissues of laying hens fed radioactive Co-Ral mixed in the feed at 100 p.p.m.

	Days on Radioactive Feed—p.p.m.[b]			Days on Normal Feed—p.p.m.[b]	
Tissue[a]	1	3	7	3	7
Blood	<0.02[c]	0.03	0.03	<0.02	<0.02
Breast	.02	.03	.02	< .02	< .02
Drumstick	< .02	.03	< .02	< .02	< .02
Fat	< .02	.03	.02	< .02	< .02
Gizzard	.05	.03	.05	.04	< .02
Kidney	.14	.15	.12	.07	< .02
Liver	.05	.09	.10	.04	< .02
Skin	.03	< .02	< .02	< .02	< .02

[a] The brain and feathers contained no acetonitrile-soluble radioactive materials during the experiment; no tissue contained acetonitrile-soluble radioactive materials after the hens were returned to normal feed for 14 and 21 days.
[b] Averaged from 3 hens sacrificed at each time interval with duplicate analysis per tissue.
[c] 0.020 p.p.m. = lower limit of sensitivity of analytical method.

Table 4.—Total p.p.m. Co-Ral equivalents and acetonitrile-solubles in eggs from hens fed radioactive Co-Ral mixed in the feed at 100 p.p.m.

Days After Treatment	Total p.p.m. Co-Ral Equivalents[a]			Acetonitrile-Solubles in Yolks
	White	Yolk	Shell	
On Radioactive Feed				
1	0.25	0.16	0.16	<0.02
2	.17	.49	.28	< .02
3	.52	.41	.72	—
4	.12	1.21	.40	< .02
5	< .02	.64	.41	—
7	< .02	2.64	.40	.03
On Normal Feed				
11	0.21	3.85	0.16	—
13	.28	2.81	.49	0.06
15	.02	1.56	.45	.07
16	.20	1.60	1.04	—
17	.12	1.04	1.20	—
18	.64	.44	.76	.03
20	< .02	.60	.36	< .02
24	< .02	.37	.24	—

[a] Total p.p.m. in 500-mg. samples of yolk, white, and shell, duplicate analysis on from 2 to 8 eggs.

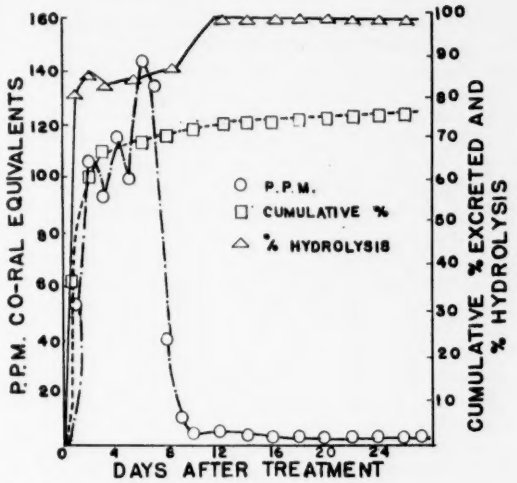

FIG. 1.—Co-Ral equivalents (p.p.m.), cumulative percentage excreted and percentage hydrolysis in the feces of laying hens fed P^{32} Co-Ral in the feed at 100 p.p.m.

from the tissues (table 2 and 3). Toxic residues were not detectable in the white of eggs.

Excretion Rates and Characterization of Fecal Metabolites.—Thirty-five per cent of the consumed P^{32} Co-Ral was excreted in the feces by the end of the first day and 66% by the second day; thereafter the amount remained at about 75% throughout the 7-day feeding period (fig. 1). The amount of radioactive materials in the feces began to decline rapidly as soon as the hens were placed on normal feed. Twenty-eight days after beginning the experiment, about 79% of the consumed P^{32} Co-Ral had been excreted in the feces. The percentage of hydrolytic products in the feces ranged from 85% to 91% throughout the 7-day feeding period (fig. 1). All P^{32} materials in the feces were hydrolytic products by 4 days after the hens were returned to normal feed as determined by chloroform:water partition coefficients.

The results of the extraction and fractionation of the P^{32} materials from the feces for Celite and anion exchange chromatography are given in table 5. From 43% to 61% of the P^{32} materials in the feces remained in the feces residue after acetone and water extraction. The acetone and water extracts were combined and partitioned with chloroform; the water-solubles constituted 31% to 34% and the chloroform-solubles 7% to 22% of the total P^{32} materials in the feces. Most of the chloroform-soluble materials co-chromatographed on Celite as Co-Ral. The O-analog of Co-Ral constituted less than 1% of the total P^{32} materials in the feces or from 2% to 8% of the chloroform-solubles. Some radioactivity in the chloroform fraction was not Co-Ral or the O-analog and was eluted from Celite in the methanol fraction (table 5).

Fractionation of the water-solubles on an anion exchange resin yielded about the same proportion of mono- and diethyl-phosphoric acid to diethyl phosphorothioic acid throughout the 7-day feeding period (table 5). The mono- and diethyl-phosphoric acids were the predominant water-soluble metabolites. These three phosphoric acids were the only ones isolated from the water-soluble products.

DISCUSSION.—Based on acetonitrile-soluble radioactivity, residues disappeared from the tissues after the hens were returned to normal feed for 7 days (table 3). However, the highest concentration of toxic residues appeared

Table 5.—Co-Ral and metabolites recovered from the feces of laying hens fed radioactive Co-Ral mixed with the feed at 100 p.p.m.

Days After Treatment	Per Cent Present as—					
	Chloroform-Celite[a]			Water-Anion[b]		Feces Residues
	Co-Ral	O-Analog	MeOH	$(C_2H_5O)_2P(O)OH$	$(C_2H_5O)_2P(S)OH$	
1	19.0	0.45	2.7	21.6	13.0	43.2
2	13.7	.47	2.7	21.1	11.7	50.4
4	5.1	.27	2.2	18.8	12.6	61.0
7	5.9	.72	2.5	19.9	13.4	57.6

[a] Identification of chloroform-solubles based on co-chromatographic behavior on Celite.
[b] Identification of water-solubles based on co-chromatographic behavior on an anion exchange resin.

in egg yolks at 11 to 15 days after treatment (table 4). The decline of residues in tissues did not correspond with the increase of residues in egg yolks. This lack of agreement in the diappearance of residues from tissues and eggs was noted when Co-Ral was dusted on poultry (Dorough *et al.* 1961), when malathion was added to the feed (March *et al.* 1956), or when Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate) was added to the feed of laying hens (Buttram & Arthur 1959). The development of an egg requires several days, and this time lag would account for the greater amount of radioactivity appearing in yolks of eggs laid several days after treatment than in eggs laid immediately after treatment. Even though good recoveries were obtained when Co-Ral was added to egg yolk (Dorough *et al.* 1961), classification of all the acetonitrile-soluble materials as Co-Ral or the oxygen analog was not justifiable. Some of the phosphoproteins, phospholipids, or other naturally occurrring phosphorus materials in egg yolks could remain in the acetonitrile fraction after partitioning with *n*-hexane and would appear as toxic residue. Certainly the nature of these acetonitrile-soluble residues needs to be more firmly established.

A large portion of the radioactive materials remained in the tissue residue after acetone and benzene extraction. The possibility that some of this radio-activity was from Co-Ral or the oxygen analog was not likely, since fractionation of the liver residue from hens dusted with Co-Ral (Dorough *et al.* 1961) yielded acid-soluble phosphorus compounds, phospholipids, ribose nucleic acid and desoxyribose nucleic acid. Complete degradation of Co-Ral to phosphoric acid and subsequent incorporation of this acid into natural phosphorus-containing metabolites is a more likely explanation than occlusion of Co-Ral or the oxygen analog in the tissue residue or protein binding of these materials.

Two methods were used in determining the percentage hydrolytic and non-hydrolytic products in the feces. The chloroform:water partitioning properties of the radioactive materials does not account for the unextractable radioactivity from the feces residue (fig. 1), whereas acetone and water extractions of the feces did account for this unextractable radioactivity (table 5). Failure to account for the unextractable radioactivity is often misleading in calculating the percentage of hydrolytic products and in calculating the percentage of each metabolite formed. For example, the oxygen analog of Co-Ral actually constituted from 2% to 8% of the chloroform-soluble materials in the feces, but when the unextractable materials were considered as an integral part of the total radioactivity, these values for the oxygen analog were reduced to 0.45% to 0.72% (table 5).

The metabolic pathway of Co-Ral fed orally to poultry was the same as that observed from dusting Co-Ral on the birds (Dorough *et al.* 1961). There was no evidence of opening of the lactone ring of Co-Ral (Krueger *et al.* 1959) by poultry and no "des-ethyl" Co-Ral was isolated from the anion exchange columns. The possibility that "des-ethyl" Co-Ral existed cannot be excluded, since O-ethyl phosphoric acid was isolated. The only unidentified chromatographic fraction from the Celite or anion exchange columns was the methanol fraction (table 5). This fraction might constitute "des-ethyl" Co-Ral, since it was found in rat excreta (Vickery & Arthur 1960).

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Influence of Caging and Transferring Techniques on Aphid Mortality and Virus Transmission[1]

PAUL P. COOK and EDWARD S. SYLVESTER[2]

ABSTRACT

Various methods of caging and transferring aphids used in the investigation of plant virus transmission were compared as to their effect on transmission efficiency and vector mortality. The methods of caging compared had no significantly measurable effect on the ability of the green peach aphid, *Myzus persicae* (Sulz.), to transmit sugar beet yellow-net virus. Darkening leaf cages by wrapping with aluminum foil did not reduce aphid mortality, nor did it increase sugar beet yellows virus transmission. None of the four methods used to transfer insects seemed to increase mortality. Survival of test aphids was found to be differentially affected by the host plant species used in the feeding sequences. Plants used were sugar beet, *Beta vulgaris* L., and smooth leaf mustard, *Brassica juncea* (Coss.).

In the development of any science, refinements in technique are essential to progress. In the study of the transmission of plant viruses by vectors, refinements in technique have paralleled the changes in sample size, experimental design, and the greater control exercised over treatment variables. Earlier methods used with aphid vectors allowed long acquisition access periods, most frequently a result of attempts to colonize diseased plants with suspected vectors. Inoculation was done by mass transfer, sometimes by simply shaking the insects off the source plant and allowing them to fall on the test plants. Long test access periods were also used, and the cage used to confine the insects near the test plant was commonly the glass chimney from a kerosene lamp. In contrast to these methods are those of the present day, especially the ones in work with aphids, in which very short, controlled, acquisition and test feeding periods (many times only a few seconds in duration) are often used to obtain transmission. Although mass transfer of vectors is still employed, individual insects are more commonly used and the test plants may be inoculated while in the cotyledon stage by single insects transferred with a microaspirator and either left uncaged or confined in a microcage with access to only a small portion of the plant. Such refinements in technique presumably have added precision to the experimental results.

Ordinarily data obtained by the several methods presently used by various workers for the rearing, transferring, and confining of aphids in the study of plant virus diseases are assumed to be comparable. Sylvester (1956) made such an assumption in discussing aphid collection by an aspirator (described below) and implied comparability with other collection methods when he stated, "This [nylon] screen acted as a resilient surface from which the aphids bounced during aspiration, and consequently mortality due to injury was negligible." However, from time to time the question has arisen as to whether such assumptions are valid. The following experiments were designed to compare some of the more commonly used methods and test whether these assumptions are justified.

MATERIALS AND METHODS.—All of the tests were done with late instar nymphs and adult apterae of the green peach aphid, *Myzus persicae* (Sulz.), reared in the greenhouse. The host plant on which the aphids were reared (either smooth leaf mustard, *Brassica juncea* [Coss.], or sugar beet, *Beta vulgaris* L.) was kept constant within a given experiment, except in those tests on mortality due to changes of food plant species. The term leaf cage used in this report refers to plastic cylinders $\frac{5}{8}$-inch in diameter and approximately $\frac{3}{4}$-inch in length, one end of which was covered with nylon net and the other end stoppered with a cork. Normally the net end of the leaf cage was placed on the leaf surface and a small board with $\frac{1}{8}$-inch thick foam rubber applied to the opposite side of the leaf, foam rubber next to the leaf; the two were held together by a rubber band. Some cages were covered with aluminum foil to within about $\frac{1}{8}$-inch of the nylon screen. This was done at the suggestion of L. L. Stubbs[3] to 1) attract the aphids to the leaf surface, and 2) reduce the amount of radiant energy absorbed by the cages. Such cages are referred to as "foiled." The collecting aspirator used consisted of the following: a $\frac{3}{16}''\times 3''$ glass tube drawn at one end to a diameter just sufficiently large to allow the aphids to pass through; the other end of the tube was inserted through a cork which fit a $\frac{9}{16}''\times 4''$ plastic tube; the opposite end of the plastic tube was covered with nylon net and inserted into a cork bored halfway through to receive it; a $\frac{3}{16}$-inch glass tube extended through the remainder of the cork and a rubber hose attached to the free end of the glass tube. The microaspirator consisted of a $\frac{3}{64}$-inch glass tube drawn to a $\frac{1}{64}$-inch tip and put in a rubber tube. The aphids, too large to enter the tube, were transferred while held by suction. Test plants were sugar beet seedlings in the cotyledon stage or with one or two true leaves.

EFFECT OF METHOD OF CONFINEMENT ON VIRUS TRANSMISSION.—*Foiled vs. Non-Foiled Leaf Cage.* Green peach aphids, which had been reared on mustard, were transferred to a sugar beet yellows source for virus acquisition. After the acquisition access period the insects were placed individually in either foiled or nonfoiled leaf cages and attached to seedling test plants. After 24 hours the leaf cages were removed and records were kept both of mortality and virus transmission. The basic test involved an intended 80 plants per treatment repeated on 16 occasions. Pretreatment mortality reduced the final number to a total of 2,349 aphids tested: 283 of 1,177 aphids in nonfoiled leaf cages and 312 of 1,172 aphids in the foil-covered leaf cages died ($\chi^2=2.04$, $P=>.10$); 56 of 1,177 aphids in nonfoiled leaf cages and 71 of 1,172 aphids in foil-covered leaf cages transmitted virus to the

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test plants ($\chi^2=2.132$, $P=>.10$). Mortality as well as virus transmission was independent of cage foiling.

Leaf Caging vs. Plant Caging.—Green peach aphids reared on a sugar beet yellow-net virus source plant and given a test feeding period individually in a leaf cage were compared with similar aphids which were given the test access period individually on plants enclosed by an inverted glass vial $1\frac{1}{8}''$ in diameter and $3\frac{1}{2}$ inches long. The test access period was 24 hours following which the cages were removed and the test plants fumigated and placed in the greenhouse for incubation. Two trials were made testing a total of 180 aphids. Sixteen of 90 aphids in leaf cages and 21 of 90 aphids in the plant cages transmitted the virus. However, the evidence for rejecting the assumption of equality of transmission was not significant ($\chi^2=0.52$, $P=<.50$).

Leaf Cages With and Without Nylon Net.—Green peach aphids reared on a large sugar beet yellow-net virus source plant were sorted singly into a series of leaf cages. One-half of the cages were stoppered with corks and fastened to test seedlings in the normal manner (nylon net between the leaf surface and the aphid). The remaining cages were fastened to the test plants with the open end against the leaf surface (no nylon net between the aphids and the leaf surface). The nylon screens of these cages were covered with aluminum foil to simulate the darkening effect of the normal cork stoppering. After a 24-hour test access period the leaf cages were removed and the plants fumigated and placed in the greenhouse. The procedure was repeated on two occasions using a total of 139 aphids. Twenty of 70 aphids in leaf cages without net and 25 of 69 aphids in regular leaf cages transmitted the virus to the test plants. The results indicated that the nylon net does not interfere with the transmission process ($\chi^2=0.62$, $P\approx.40$).

Mass Acquisition in Large Leaf Cages vs. Plastic Tube Enclosure.—A group of green peach aphids reared on nondiseased mustard was placed in a single large leaf cage ($1\frac{1}{8}$ inches diameter$\times 1\frac{5}{8}$ inches long) and attached to a leaf of a sugar beet yellow-net source plant. A second group was placed in a plastic tube ($2\frac{1}{2}''\times 6\frac{1}{2}''$) one end of which was capped with muslin. Ventilation was increased by a muslin-covered opening ($1''\times 1\frac{1}{2}''$) near the base of the cage. The cage was inverted over a sugar beet yellow-net source plant. A 1-day acquisition access period was allowed for both groups. The aphids were then placed singly on test plants and confined by an inverted glass vial for 1 day, after which the vials were removed and the plants fumigated and placed in the greenhouse. The procedure was done on three occasions testing a total of 224 aphids. Twenty-one of 110 aphids allowed acquisition access in large leaf cages, and 28 of 124 aphids given acquisition access under plastic tubes, transmitted virus to the test plants. Free access to the virus source plant did not appear to increase vector inoculativity over an acquisition access period in a leaf cage ($\chi^2=0.242$, $P\approx.60$).

EFFECT OF METHOD OF TRANSFER ON APHID MORTALITY.—Four methods of transferring aphids were compared for their effect on mortality. The method hereafter designated as BB consisted of collecting the aphids singly with a No. 1 camel's-hair brush moistened with saliva, and placing them directly in leaf cages. In the method designated KB the aphids were knocked off the host plant onto a paper or cloth by jarring the leaf, and picked up with a brush and placed in the leaf cages. Other aphids were collected with an aspirator, tapped out onto a paper or cloth, and picked up with a microaspirator and placed in leaf cages; this method is designated AM. The fourth method, MM, was to collect the aphids from the colony plant singly with a microaspirator and place them directly into leaf cages. All aphids were reared on mustard and were confined individually in leaf cages. The leaf cages were placed in the laboratory with no opportunity for the aphids to feed. The mortality reading was made after 24 hours. Eight replications were run using a total of 800 aphids. The following results were obtained: in BB, 136 of 200 aphids were dead after 24 hours; KB, 138 of 200; AM, 145 of 200; MM, 126 of 200. Mortality appeared to be independent of the treatments ($\chi^2=4.246$, d.f. $=3$, $P\approx.25$).

EFFECT OF HOST PLANT SPECIES ON APHID MORTALITY.—Aphids were reared and fed on various sequences of mustard and sugar beet in an attempt to determine if the host plant influenced mortality. Four replications were run each using approximately 100 aphids reared on mustard. Fifty aphids were transferred to mustard and 50 to sugar beet. After 24 hours mortality was recorded and the survivors in each group were divided again between mustard and beets. After another 24 hours mortality was again recorded and the aphids discarded. Four similar trials were also made using aphids reared on sugar beet. Thus eight feeding sequences were tested using all possible combinations of the two hosts in series of three, and testing a total of 796 aphids. In the following, "m" refers to mustard and "b" refers to sugar beet. The first letter of a group (*e.g.*, mbm) indicates the host on which a group of aphids was reared, the second refers to the host fed on for the first 24 hours of the test, and the third refers to the host fed on for the final 24-hour period. The results are given in table 1.

Aphids reared on mustard and those reared on sugar beets were not compared experimentally, since the tests were conducted at different times of the year. The experiments with the former were concluded in March and with the latter in May. However, it is of interest to

Table 1.—Results[a] of trials to determine the effect of changing host plant species on green peach aphid mortality.

Source Plant	Aphid Mortality			
	24-Hour		48-Hour	
Mustard	mm[b]	11/199	mmm	11/94
			mmb	8/94
	mb	17/200	mbm	20/91
			mbb	17/92
Beet	bm	26/197	bmm	12/85
			bmb	22/86
	bb	33/199	bbm	21/83
			bbb	31/83

[a] In ratios listed, numerator is the number of aphids dead; denominator is the number counted.

[b] See text for explanation of symbols. Briefly, mmb means the sequence of transfer was from mustard to mustard to beet.

Table 2.—Influence of varying the amount of sugar beet and mustard in a feeding sequence on the mortality of green peach aphids.

Host Plant	Aphid Mortality According to Amount of Host in Transfer Sequence					
	+++[a]		++		+	
	Number[b]	Per Cent	Number	Per Cent	Number	Per Cent
Mustard	11/94	11.7	28/185	15.1	17/92	18.4
Beet	31/83	37.3	43/169	25.4	12/85	14.1

[a] A "+" indicates a feeding period, either on the colony plant where aphids were reared, or on a plant fed upon for one of the 24-hour periods.

[b] In ratios listed, numerator is number of aphids dead; denominator, number observed.

compare the number of times the aphids were exposed to mustard or sugar beet and the accompanying mortality data (see table 2). The results clearly indicated that mustard was the more favored host, in as far as survival was concerned, since aphids from mustard had an increase in mortality as feeding on sugar beets increased, while those from sugar beet had a decrease in mortality as feeding on mustard increased.

Statistical comparison of mortality at the end of the first 24-hour period shows no significant evidence for a difference between mm and mb ($\chi^2 = 0.960$, $P \approx .30$), or between bm and bb ($\chi^2 = 0.70$, $P \approx .30$). Analyses of the 48-hour data, however, indicate greater differences in mortality than would be expected to occur by chance, and therefore support the conclusion reached above that mustard is the more favored host species as far as aphid survival is concerned. For mmm, mmb, mbm, and mbb, $\chi^2 = 7.37$, d.f. = 3, $P \approx .02$; for bmm, bmb, bbm, and bbb, $\chi^2 = 12.96$, $P \approx .01$.

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Inhibition of House Fly Ali-Esterase and Cholinesterase Under *in vivo* Conditions by Parathion and Malathion[1]

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Abstract

Ali-esterase (Ali-E) in adult female house flies (*Musca domestica* L.) was rapidly inhibited *in vivo* following treatment with parathion or malathion. When dosages were such that some or all of the insects survived the insecticide treatment, levels of Ali-E activity returned to normal within 24 hours. The observed patterns of inhibition were similar with both insecticides and did not appear to be directly related to their toxic action. Cholinesterase activity was more slowly and less reversibly inhibited and the inhibition was closely correlated with the observed symptoms of poisoning. Patterns of inhibition of both esterases were similar in strains of flies resistant and susceptible to parathion.

Van Asperen (1958) demonstrated that in house flies (*Musca domestica* L.) poisoned by exposure to vapors of DDVP, aliphatic esterase (Ali-E) activity of body homogenates was more inhibited at the time of knockdown than was the acetyl-cholinesterase of head homogenates. More recently it has been shown (Van Asperen & Oppenoorth 1959, Bigley & Plapp 1960) that Ali-E levels are much lower in organophosphorus-resistant strains of house flies than in susceptible strains, whereas there are no significant changes in either levels or sensitivity to inhibition of cholinesterase (ChE) activity. This finding must be regarded as of extreme interest in studying the mode of action of organophosphorus insecticides and the changes that occur in insects that develop resistance.

The study reported provides information on the relative susceptibility of Ali-E and ChE activity of house flies to inhibition by parathion and malathion under *in vivo* conditions at different levels and with several methods of treatment. In previous studies it has been shown that with parathion, Ali-E activity is four times more susceptible to inhibition *in vitro* than ChE, whereas with malathion, ChE is twice as susceptible to inhibition as Ali-E (Bigley & Plapp 1960).

Data are also presented on the comparative inhibition of the two enzymes by parathion in susceptible and resistant flies, and on the importance of the substrate protection technique described by Van Asperen in measuring esterase inhibition by parathion.

Methods.—*Test Insects.*—Two- to four-day-old adult female house flies were used in the experiments described in this paper. The susceptible flies were from the Orlando Regular Colony and the organophosphorus-resistant flies were from the Tropical P Colony, a parathion-resistant strain (Wilson *et al.* 1959).[2] When these experiments were run, the Tropical P Colony was about five times less susceptible to parathion than the Orlando Regular (susceptible) Colony, a difference based on mortalities obtained by topical application of the insecticide. Resistance to para-oxon in the Tropical P Colony was of the same order of magnitude as resistance to parathion. Ali-E activity in the resistant strain, based on hydrolysis of methyl butyrate by body homogenates, was about 60% of that shown by the susceptible strain.

Unless otherwise specified, flies were treated by topical application with 1 μl. of acetone solutions of the insecticides to the tip of the abdomen. Treated insects were held at a temperature of approximately 80° F. and 60%–70% relative humidity. At the times samples were taken the flies were anesthetized with carbon dioxide, dipped

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[2] Both colonies were obtained from the Orlando, Florida laboratory of the Entomology Research Division, U. S. Department of Agriculture.

in three acetone rinses to remove any unabsorbed insecticide, and decapitated. ChE determinations were made on head breis and Ali-E determinations on body (thorax plus abdomen) breis. Samples were held on ice until analyses were performed. Each sample was homogenized immediately prior to analysis. With the exception of a few 8- and 12-hour samples, all determinations of enzyme activity were made within 4 hours of the time the insects were sampled. Holding up to 12 hours did not appear to decrease ChE activity in collected flies, but Ali-E activity was markedly less within a few hours. Control flies were treated with acetone and handled in the same manner as those treated with insecticide.

Measurements of Cholinesterase and Ali-esterase Activity.—The colorimetric method of Hestrin modified by Robbins *et al.* (1958) was used throughout these tests to determine enzyme activity. The reagents and their concentrations have been previously described (Bigley & Plapp 1960). Unless otherwise specified, the substrate protection technique was employed. Fly head ChE and fly body Ali-E activity were determined using acetylcholine bromide (2.5×10^{-3} M) and methyl *n*-butyrate (4×10^{-3} M), respectively, as substrates. Samples of insect tissue for analysis were homogenized in equal volumes of substrate and phosphate buffer (pH 7.2) in Potter-Elvehjem glass homogenizers. The homogenates were filtered through glass wool and the particulate material discarded. Two ml. of the prepared homogenate containing 1 ml. of substrate and the equivalent of either one fly head or two fly bodies were pipetted into test tubes containing 1 ml. of distilled water. They were then incubated at 37° C. for 15 minutes, prior to the addition of hydroxylamine, which quenched the reaction. Both standards (untreated flies) and treated samples were prepared in this manner. Controls (no hydrolysis of substrate) were prepared by pipetting aliquots of head or body homogenates directly into tubes containing hydroxylamine. The difference between standard and control readings on the spectrophotometer at 540 mμ. was considered to be normal enzyme activity. Activity in the various samples of treated flies was expressed as per cent of normal enzyme activity.

Insecticides.—Technical grade parathion and malathion were used. For most of the studies with parathion, samples of the insecticide were incubated overnight in phosphate buffer to remove any possible traces of more powerful esterase-inhibiting impurities (Metcalf 1959). The sample of malathion was of greater than 95% purity and was used without further purification.

EXPERIMENTAL.—*Protection as a Factor in Measuring Enzyme Activity.*—Recent work by Van Asperen has indicated that some estimates of ChE inhibition in house flies poisoned with organophosphorus compounds may be too high. This author found that homogenizing samples of treated flies containing unmetabolized insecticide brought additional quantities of inhibitor into contact with the enzyme during the process of sample preparation and thus gave an artificially high value of inhibition. To prevent this, he homogenized his samples in substrate, the substrate protecting the enzyme from further inhibition. DDVP, the insecticide used in that study, is, of course, a powerful enzyme inhibitor *per se*.

The importance of the protection technique was measured in several experiments. In one, flies were treated with an LD-50 of parathion, and ChE and Ali-E activity were determined at various times after treatment with and without protection. The protection technique was identical to that described in all *in vivo* experiments. The comparable nonprotection technique (Seume *et al.* 1960) consisted of homogenizing flies in buffer only and then adding substrate to aliquots of the homogenate at the time sample incubation began.

Table 1.—Protection vs. nonprotection in measuring inhibition of ChE and Ali-E in susceptible house flies treated with an LD-50 of parathion—(0.6 microgram per gram)

Time After Treatment (minutes)	Per Cent Normal Enzyme Activity			
	ChE		Ali-E	
	Protection	Nonprotection	Protection	Nonprotection
15	96 ± 1[a]	102 ± 4	25 ± 2	2 ± 0
30	99 ± 2	91 ± 3	21 ± 3	6 ± 3
60	95 ± 1	101 ± 0	19 ± 3	14 ± 6
120	91 ± 3	93 ± 4	31 ± 3	24 ± 6
240	67 ± 1	65 ± 2	29 ± 5	33 ± 4
720	46 ± 2	53 ± 3	—	—
1,440	75 ± 3	85 ± 4	—	—

[a] Standard deviation.

The results are given in table 1. Standard deviations are shown here to illustrate typical amounts of variability. With parathion, ChE activity was about the same by both methods of analysis. With Ali-E, protection resulted in definitely lower inhibition values in the early posttreatment samples at such times as when maximum concentrations of insecticide are present in treated insects. By 2 hours after treatment, when most of the applied insecticide had been metabolized (Plapp *et al.* unpublished data) there were few differences in the inhibition values obtained by the two methods.

In another experiment flies were treated with parathion from one-fourth to four times an LD-50. At the higher dosages flies were sampled at knockdown; at dosages less than LD-50 they were sampled at the same time as at the LD-50, about 3 hours after treatment. ChE and Ali-E activity were measured as described before, with and without protection.

The results were in substantial agreement with those in the previous experiment. Here, also, the protection technique failed to give lower estimates of ChE inhibition, regardless of the amount of insecticide used. As in the previous experiment, the protection technique resulted in considerably lower estimates of Ali-E inhibition at all dosages employed.

The results obtained by comparing the protection and nonprotection techniques are in agreement with those of Colhoun (1959) and Seume *et al.* (1960). Colhoun reported that protection made little difference in measuring ChE inhibition in cockroach nerve cord due to parathion, but that the technique was necessary in measuring inhibition due to para-oxon. Seume *et al.* found that with parathion, protection made no differences in estimates of inhibition

of rat brain ChE, but did result in lower estimates of inhibition of enzymatic hydrolysis of *o*-nitrophenyl acetate. Because of the fact that protection resulted in lower estimates of Ali-E inhibition, this technique was explored in all further studies.

Patterns of ChE and Ali-E Inhibition by Parathion and Malathion.—The inhibition of ChE and Ali-E due to treatment of flies with different dosages of parathion or malathion was determined as a function of time after treatment. In these experiments the general method of Mengle & Casida (1958) was followed. Flies were treated with amounts of insecticide that gave about 50% mortality. This method allowed measurements of the recovery of the inhibited enzymes in flies surviving treatment. Flies were also treated with twice the LD-50 of both insecticides, which gave a 95% to 100% mortality. In other experiments, flies were treated with five times an LD-50 of parathion and with an amount of malathion that gave a 25% mortality. Measurements of enzyme activity with LD-50 or lower dosages were made at 15, 30, 60, 120, 240, and 1440 minutes after treatment. With the higher dosages, determinations were made at 5, 10, 20, 40, 80, and 160 minutes after treatment. With both insecticides maximum knockdown due to treatment with an LD-50 occurred after 3 to 4 hours. With lethal dosages of parathion, knockdown occurred within 80 minutes of treatment, and with malathion, between 80 and 160 minutes.

At the indicated times, symptoms of poisoning in treated flies (normal, hyperactive, or paralyzed) were recorded, and samples collected and prepared for analysis as previously described. In all the experiments described here, ChE and Ali-E activity were determined in the same flies, ChE in head breis and Ali-E in body breis. The method allowed a direct comparison of the inhibition of both enzymes on the same sample of insects.

The results are shown in figure 1. In all experiments, maximum inhibition of Ali-E activity occurred almost immediately after treatment. Maximum inhibition varied from 45% with an LD-50 of parathion to 87% with twice an LD-50 of malathion. By the time treated flies showed visible symptoms of poisoning, such as hyperactivity and loss of coordination, inhibition of Ali-E activity was always less than the maximum, usually about 50% of normal at the time the maximum number of deaths occurred. ChE inhibition occurred more slowly than Ali-E inhibition and was more closely associated with the symptoms of poisoning. Flies treated with an LD-50 of both compounds showed signs of poisoning only when ChE activity fell below 70% of normal. At higher levels of treatment, however, definite symptoms of poisoning were noted before much ChE inhibition occurred. Nevertheless, inhibition of ChE activity was more closely correlated with the symptomology induced by poisoning, maximum inhibition always occurring at or near the time of knockdown. With malathion the maximum ChE inhibition was between 50% and 60%. With an LD-50 of parathion, ChE was 69% inhibited 4 hours after treatment. Treatment with five times an LD-50 was necessary to produce a 90% inhibition of ChE activity, indicating that complete inhibition of ChE was not necessary to produce toxicity.

The results also indicated a greater reversibility of Ali-E inhibition when dosages were such that treated flies survived. Ali-E activity was always near normal levels 24 hours after treatment, whereas complete recovery of ChE activity never occurred. This lack of recovery of ChE activity in treated flies differs from the results of Mengle & Casida (1958), which indicated that with parathion, malathion, and a number of other phosphorus insecticides, ChE activity in survivors was at nearly normal levels 1280 minutes after treatment with an LD-50. It appeared that the greater recovery of Ali-E was due to actual recovery of the affected enzyme rather than synthesis of new enzyme.

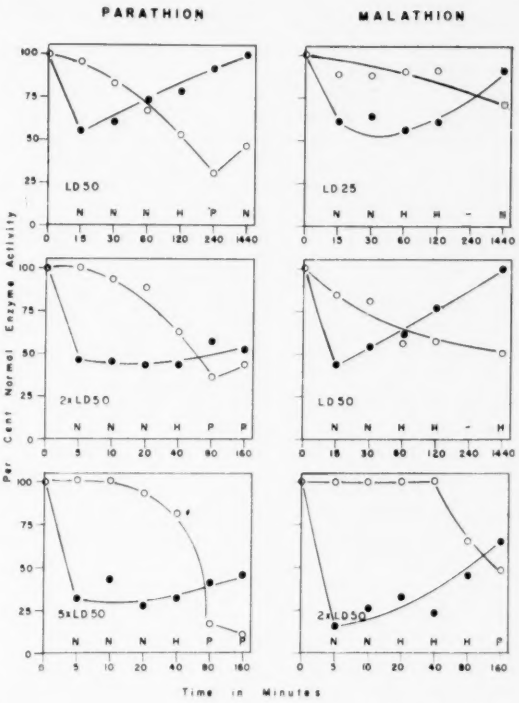

FIG. 1.—Symptomology and inhibition of ChE and Ali-E in susceptible house flies treated topically with various dosages of parathion or malathion. LD-50 of parathion, 0.6 microgram per gram; LD-25 of malathion, 10 micrograms per gram; LD-50 dose, 15 micrograms per gram. Symptomology: N = flies normal, H = hyperactive, P = paralyzed. ○——○ = ChE, ●——● = Ali-E.

Effect of Varying the Method of Treatment on ChE and Ali-E Inhibition.—In the previously described experiments flies were treated with the insecticides by application of acetone solutions of the toxicants to the tip of the abdomen. With this method, the extremely rapid inhibition of Ali-E activity indicated that the effect of the toxicants on this enzyme *in vivo* may have been due to proximity to the site of treatment, rather than greater susceptibility. To explore this possibility, two experiments were run. In one, flies were treated with parathion by topical application of acetone solutions of the insecticide to the head. In a second experiment, flies were treated

with malathion by exposure to insecticide films in glass jars. The films were prepared by pipetting the required amount of insecticide (12 μg.) for an LD-50 into pint jars and then rotating the jars to form a film over their entire inner surfaces. One hour later, 20 flies were placed in each of the treated jars. Both experiments were run at dosages of insecticide that gave only partial mortality, and all analyses were performed as before, utilizing the substrate protection technique.

The results are presented in table 2. Inhibition of Ali-E activity was markedly delayed when compared with the previously described topical-abdominal treatments, but, as before, it occurred before ChE activity was inhibited and was returning to normal levels 24 hours after treatment.

Table 2.—Effects of varying the method of treatment with parathion and malathion on patterns of ChE and Ali-E inhibition in susceptible house flies.

Time After Treatment (minutes)	Parathion—Topical Treatment (on head): Per Cent Normal Enzyme Activity			Malathion—Exposure to Film in Glass Jars: Per Cent Normal Enzyme Activity		
	ChE	Ali-E	Symptoms	ChE	Ali-E	Symptoms
15	90	100	N[a]	80	58	N
30	99	79	N	96	50	N
60	106	60	N	95	48	N
120	81	66	H	68	45	H
240	78	72	H	39	43	P
480	73	56	H	34	105	P
720	69	74	H	51	108	H
1,440	87	77	N	67	107	H

[a] N = Normal, H = Hyperactive, P = Paralyzed.

Treatment by both of the methods used here also caused a delay in the onset of ChE inhibition. The dosage of parathion gave only a 30% mortality and caused only about a 30% reduction in ChE activity. With malathion, a 60% to 65% inhibition of ChE activity occurred at both 4 and 8 hours after treatment.

Ali-E inhibition occurred more rapidly than ChE inhibition in flies treated with both insecticides regardless of the method of application. However, as in the previous experiments, inhibition of ChE activity was more closely correlated with the symptomology of poisoning induced by the insecticides.

Effects of Cumulative Dosages of Parathion.—It is known that a number of organophosphorus insecticides are cumulative in their effects on insects; that is, repeated treatment with sublethal dosages will cause mortality. It should not be assumed that insects do not detoxify the insecticide, but rather that the inhibition of enzymes involved in the poisoning process is essentially irreversible and when enough of the vital enzyme or enzymes is inhibited the insects will die.

Different experiments performed in this study indicated that Ali-E activity is inhibited by sublethal treatments with both parathion and malathion. The inhibition appeared to be quite reversible; and, as shown in figure 1, recovery of Ali-E activity was essentially complete within 24 hours. ChE, on the other hand, was not much inhibited by sublethal dosages; substantial inhibition occurred only when symptoms of poisoning were manifested. At the same time, ChE inhibition was apparently less reversible; complete recovery of inhibited ChE did not occur in 24 hours in flies surviving an LD-50.

An experiment was set up to measure the effect of treatment with a series of sublethal dosages of parathion on the inhibition of the two enzymes. Two-day-old flies were treated with parathion at a dosage level that caused partial mortality within 24 hours and with a series of smaller dosages. The surviving flies at each dosage level were retreated daily with the original dosage of insecticide until death occurred. Samples of flies were collected 1 and 24 hours after treatment and ChE and Ali-E activity determined. The procedure was repeated daily until all the flies died. Several times, when it became obvious that the flies treated at a particular level were near death, additional samples were collected for enzyme determinations.

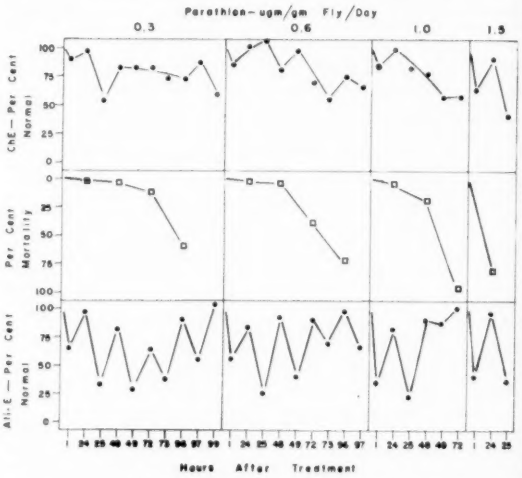

FIG. 2.—Inhibition of ChE and Ali-E and mortality in susceptible house flies caused by repeated daily treatment with sublethal dosages of parathion.

The results are shown in figure 2. It was assumed when the experiment was started that the samples taken 1 hour after treatment would show higher inhibition of the more susceptible Ali-E, and that th samples taken 24 hours after treatment would show lower recoveries of the apparently more irreversibly inhibited ChE. This was found to be so. The daily variation between Ali-E inhibition and recovery was much greater than that of ChE. Ali-E was greatly inhibited by all the insecticide treatments, including those which failed to cause mortality. As shown, particularly at the lower dosages, maximum inhibition usually occurred the day before maximum mortality. By the time the flies died, the susceptibility of this enxyme to the insecticide appeared to be decreased.

With ChE there appeared to be less recovery of the enzyme from day to day. This was most notable with the higher levels of treatment and at the lower dosages several days after the initial treatment. As in previous experiments, inhibition was most marked in flies showing

Table 3.—ChE and Ali-E inhibition in parathion-resistant house flies treated topically with an LD-50 of parathion (3 micrograms per gram).

Time After Treatment (minutes)	Per Cent Normal Enzyme Activity		Symptoms
	ChE	Ali-E	
15	82	9	N[a]
30	65	23	H
60	34	30	P
120	45	33	P
240	71	81	H
1,440	80	89	N

[a] N = Normal, H = Hyperactive, P = Paralyzed.

symptoms of poisoning and greatest at the time of death. However, it should be noted that the total inhibition of ChE did not reach very high levels; a 50% inhibition occurred only with the highest treatment level. This may be contrasted with the 60% to 70% inhibition in flies treated with an LD-50 or two LD-50's of parathion as shown in figure 1.

Comparisons between Flies Resistant and Susceptible to Parathion.—Results by Bigley & Plapp (1960) demonstrated that ChE activity of the susceptible and resistant strains was present in nearly identical amounts and was equally susceptible *in vitro* to parathion. Ali-E in both colonies was also equally susceptible to inhibition by parathion, about five times more so than ChE, although there was considerably less Ali-E in the resistant strain.

When the two colonies were compared, the problem arose as to whether flies should be treated with the same effective amount of insecticide, such as an LD-50, or with the same total amount. Comparisons of both types were made. In one experiment, flies of the resistant strain were treated with an LD-50 of parathion five times that needed for 50% mortality with the susceptible colony. The results are shown in table 3. Ninety per cent inhibition of Ali-E activity was found in the resistant strain 15 minutes after treatment. As in all other experiments inhibition was decreasing by the time the flies showed symptoms of poisoning. ChE activity was inhibited almost as much as with susceptible flies, and maximum inhibition also occurred at the time of knockdown. The only difference noted was that both knockdown and maximum ChE inhibition with the resistant flies occurred more rapidly than with susceptible flies treated at the LD-50 level. Both in this and other experiments dosages of parathion lethal to both colonies affected the resistant flies as rapidly as the susceptible flies.

In another experiment, flies of both colonies were treated with parathion at the rate of 50 micrograms per gram, and the inhibition of ChE and Ali-E were measured at different times until death. The amount of insecticide used was far in excess of that required to produce mortality in either colony.

The results are shown in figure 3. The rate of knockdown and the pattern of ChE inhibition were almost identical in the two colonies. ChE activity was more than 85% inhibited in both. The initial inhibition of Ali-E was similar. With the susceptible colony, only slight recovery of the enzyme occurred before death, whereas with the

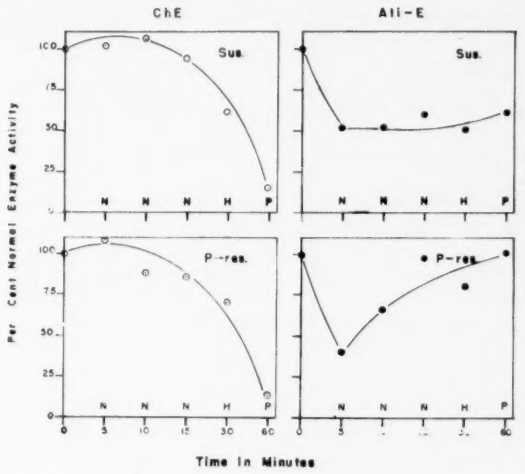

FIG. 3.—Symptomology and inhibition of ChE and Ali-E in parathion-resistant and -susceptible house flies treated topically with a massive dosage of parathion (50 micrograms per gram). Symptomology: N = flies normal, H = hyperactive, P = paralyzed.

resistant flies, inhibition appeared to be rapidly reversed, and Ali-E activity reached normal levels by the time of death.

DISCUSSION.—The experiments described in the study reported herein showed that with both parathion and malathion, Ali-E activity was more rapidly inhibited than ChE under *in vivo* conditions. This was true in both susceptible and parathion-resistant strains of flies and under all test conditions.

At the same time, the results showed that inhibition of Ali-E did not appear to be correlated with the toxic action of the insecticides. Ali-E activity was always inhibited before symptoms of poisoning were observed and was usually recovering from the early massive inhibition at death. In both the cumulative-dosage experiment and in the experiment in which resistant flies were treated with a massive dose of parathion (50 micrograms per gram) almost complete recovery of Ali-E had occurred at death.

ChE inhibition, on the other hand, was always closely related to the symptomology of poisoning. This enzyme was only slightly inhibited by sublethal dosages of both insecticides, whereas a 50% to 70% inhibition occurred at LD-50 treatment levels. Maximum inhibition was always closely correlated with the time of knockdown. The much lower reversibility of ChE inhibition in the cumulative-dosage experiment was probably sufficient to explain the toxic effects of repeated sublethal dosages of parathion.

Another interesting pattern was noted. With both insecticides Ali-E activity was usually inhibited to the same degree regardless of the amount of insecticide. Thus, lethal dosages caused no more inhibition than LD-50 or sublethal dosages. The inhibition of ChE activity was always more closely related to the amount of insecticide used. Sublethal dosages caused little or no inhibition, LD-50's caused partial inhibition, and massive amounts

caused almost complete inhibition. If it is assumed that ChE inhibition is directly related to toxicity, then it must be inferred that complete inhibition is not necessary to cause mortality, since only massive dosages of insecticide caused a high degree of enzyme inhibition. At the same time, it is necessary to remember that at sites other than the brain, much greater ChE inhibition may have occurred.

Nevertheless, a number of independently selected oganophosphorus-resistant strains of house flies have shown low Ali-E activity and there is little doubt that a physiological relationship exists between low levels of Ali-E activity and resistance. Since resistance is usually assumed to be caused by an increased ability of the resistant insect to detoxify the insecticide, it would follow that the occurrence of less of an enzyme could not be expected to be the factor responsible. Recently Van Asperen & Oppenoorth (1960) have presented an explanation for this apparently anomalous situation by demonstrating that in resistant strains the Ali-E is apparently modified to a "phosphatase" capable of breaking down the phosphate analogs of phosphorothioate insecticides.

However, we feel that another explanation is possible. The data presented in this paper shows that Ali-E in both resistant and susceptible strains is inhibited *in vivo* at an extremely rapid rate. At the same time, recovery of the enzyme is also rapid in both strains of flies studied, although it appears to occur more rapidly in the resistant strain. If the inhibition of Ali-E is a binding effect rather than an irreversible phosphorylation of the enzyme as is the case with ChE, then Ali-E may be involved in poisoning by serving as a temporary storage site for the insecticide and it is possible that the insecticide molecule is released intact from the enzyme surface. Other enzymes involved in detoxication may eventually be rendered ineffective, either due to phosphorylation or accumulation of excessive amounts of their normal substrates. A release of insecticide from the Ali-E could at such a time allow the insecticide to be transferred to ChE where it then exerts its toxic effect. The lower levels of the enzyme in resistant strains could then serve simply as a less effective storage site for the insecticide. The results presented in this paper provide a basis for this assumption as in almost all our experiments, a decrease of Ali-E inhibition took place before poisoning occurred. Final elucidation of the importance of Ali-E in insect resistance to organophosphorus insecticides must await studies on the purification of the enzyme, the nature of its reaction with the toxicant, and its function in the organism.

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An Ecological Approach to the Insecticide Problem[1]

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ABSTRACT

Insecticides effected important changes in the structure and physiology of the *Brassica oleracea* L. (Cruciferae) community. The number of species in some insecticide-treated communities were reduced to one-half, relative to the control community. Insecticides severely reduced the numbers of many species populations, but with some insect populations the insecticides caused density increases and in a few cases the populations increased 10-fold. The significant increase in pest populations resulted in most instances from insecticide destruction of natural enemies. Insect control without the usual complications of outbreaks, resistance, and residues is possible if the ecology of populations and communities is thoroughly understood and then insecticides are selectively applied.

Both the structure and physiology of communities can be seriously affected by applications of poisonous chemicals. Disturbances to natural communities caused by insecticides such as DDT are well documented. When insecticides are used properly, they act more as a medicine than a poison for the community; however, like medicine, improper use often results in injury. Whether an insecticide is helpful or harmful depends primarily on how the material is employed. Reviews of the problems

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caused by insecticides are well presented by Pickett & Patterson (1953), Ripper (1956), and Menhinick (1960).

Because communities have a structure and a physiology, they should be studied as a whole. This approach has been neglected in most insect control studies. The attention of the present investigation was focused on the *Brassica oleracea* L. (Cruciferae) community and how insecticides influenced its structure and physiology.

METHODS.—Based on what is already known about insecticides and the ecology of communities, some changes in the structure and physiology of specific populations were expected when insecticides were introduced into the community. To detect such changes in the structure and/or physiology of the community these experiments were designed to reveal and make possible the study of the community as an organized unit.

The animal community associated with *Brassica oleracea* was selected because members of this community are abundant in both numbers of species and individuals (Pimentel 1960). In these experiments single large communities were studied instead of replicates of several adjacent smaller communities, because the large communities were known to be more stable and would provide a more exact picture of differences between the communities and their treatments. The plots measured 75 feet by 100 feet, and they were separated from one another a minimum of 500 feet to reduce the effects caused by the movements of animals between experimental communities. In addition, the plots were laid out perpendicular to the prevailing northwest wind to reduce chance transport of insects from community to community.

During the 1957 season, five-row blocks consisting of equal numbers of the following *B. oleracea* varieties were planted in each plot: broccoli, Brussels sprouts, cabbage, collards, and kale. In 1958, when the control community was replicated for a further check on variation, only collards were planted. The flat open leaves facilitate insect counting, and there is little difference in insect abundance on collards and the other varieties. Both collards and broccoli blossom readily and provide nectar for the insects (Lepidoptera, Hymenoptera, *et al.*) which require nectar.

All plants were 6 weeks of age at the start of the experiments. The plots were fertilized with a 5-10-5 fertilizer applied at a rate of 2,000 pounds per acre; they were limed at a similar rate.

The control community, referred to as "control," received no insecticide. The community, identified as "DDT," received DDT in the quantities listed in table 1. The community receiving a scheduled treatment of rotenone is referred to as "rotenone" (table 1). The community receiving an insecticide treatment of endrin, parathion, and malathion as recommended by extension specialists for cabbage pests is identified as "parathion" (table 1).

Commencing the last week of May and thereafter weekly for 11 weeks, 100 plants were measured and animal populations sampled on the 100 plants in each plot. The frequent sampling over a long period of time reduced variability caused by changes in plants, temperature, and moisture. Density changes were recorded for more than one species to add support to any general trends. The same investigator did all of the sampling in order to reduce variation caused by individual differences in sampling. The plants were selected systematically to insure a representative sample of the entire plot. By systematically selecting the 100 plants from the total planting, it was believed that no bias was introduced. The outer rows were excluded from any sample because of the possibility of edge effects. An estimate of the total surface area was made by placing a sheet of plastic, which had been etched in 1-square-inch squares, against each leaf and estimating the number of square inches of surface area on the plant. Only one side of the leaf was included in the measurement. The area estimated in this way was demonstrated to have an error of less than 10%.

Table 1.—Treatment schedules of communities which received insecticides.

COMMUNITY	DATE	INSECTICIDE	RATE (LB./A.)
DDT[a]	May 24	DDT	0.25
	June 10	DDT	.25
	July 1	DDT	.25
	July 9	DDT	.25
	July 16	DDT	.25
	Aug. 5	DDT	.25
Rotenone[b]	May 30	Rotenone	0.07
	June 26	Rotenone	.07
	July 1	Rotenone	.07
	July 9	Rotenone	.07
	July 15	Rotenone	.07
	July 25	Rotenone	.07
	Aug. 6	Rotenone	.07
Parathion[a]	May 24	Endrin+parathion	0.18+0.20
	June 26	Endrin+parathion	.18+0.20
	July 10	Endrin+parathion	.18+0.20
	July 19	Endrin+parathion	.18+0.20
	Aug. 6	Malathion	.64

[a] Emulsion.
[b] Dust.

Sampling the populations in the field was difficult, and in some cases it was not possible to distinguish between species, genera, and families. For this reason the organisms were classed into taxa, which could be accurately determined in the field. A list of the taxa and a discussion of the ecological role of each will be presented in another paper (Pimentel 1961). The method of grouping species for counting reduced the categories of taxa in this study to one-fourth of the number expected according to the standard obtained in the initial survey. In estimating the number of individuals of any taxon, only a single stage in the life history of each taxon was used. The stages selected were those that would give the best estimate of the density of the species and of parasitism rates in the species. The following is a list of the types found and the methods by which their densities were determined:

Lepidoptera.—Members of this order were counted in the last larval instar. As the larvae were counted, they were collected and placed into separately labeled vials and then taken to the laboratory where they were held to determine rates of parasitism and disease. The pupal stages were not included in the count, but they were col-

lected and held to record any parasites not commonly found attacking the larvae.

Homoptera.—Members of this order were counted in the adult stage. Those Aphididae which were parasitized and attached to the plant were counted as parasitized. Aphids from which parasites had emerged were not counted. When more than 100 were present, a sample of these was taken; otherwise, all parasitized aphids were taken to the laboratory for holding to determine species of parasites and their numbers.

Hemiptera.—Members of this order were counted in the adult stage. Parasitism was recorded when observed.

Coleoptera.—Members of this order were counted in the adult stage. Last stage larvae were collected and held for the emergence of parasites.

Diptera.—*Liriomyza*, *Scaptomyza*, and *Aphidoletes* groups were counted in the third larval instar. Pupae were held for parasite emergence. The Syrphidae were counted in the pupal stage and held to determine parasites and rates of parasitism. All remaining Diptera were counted in the adult stage, and the larvae when seen were collected to determine rates of parasitism.

Hymenoptera.—Members of this order, except those species belonging to the family Formicidae, were counted as so many parasitic attacks upon their hosts, *i.e.*, 10 infested hosts were recorded as 10 parasites of some species. Hosts were collected in the field and held for parasite emergence. Rates of parasitism in this order were determined where possible.

Neuroptera.—Members of this order were counted in the last larval stage, and they were held in the laboratory to determine species and rates of parasitism.

Arachnida.—All stages were counted, and rates of parasitism determined where possible.

Other Macroscopic Animals.—Any representative in this category was counted in the adult stage, and parasitism was determined where possible.

Microorganisms.—Members of this group were counted as so many individual animal host infections.

In order to compensate for the increasing plant size as the season progressed, the taxa densities were adjusted to a constant leaf surface area of 20,000 square inches.

RESULTS.—The taxa present in the control, DDT, rotenone, and parathion communities differed (table 2). The data giving a complete list of the taxa observed in each of the experimental plots and the number of individuals counted per week are filed in the Comstock Memorial Library of Cornell University. Anyone interested in these data may obtain copies on interlibrary loan.

The control community had the largest number of taxa, followed in order by the DDT, rotenone, and parathion communities. There was little difference between the herbivorous taxa in the control, DDT, and rotenone communities; 44% to 46% of the total taxa were herbivorous (table 2). Parasitic and predaceous taxa were practically extinct in the parathion community, although its herbivorous taxa remained similar to the other plots. A total of 78% of the taxa in the parathion community was herbivorous compared with the 44% to 46% recorded in the other communities. Why were there so many carnivorous taxa in the plots that were receiving lethal doses of these insecticides? The answer lies in the number of herbivores present. In spite of the high mortality of carnivores observed in these communities, the herbivore populations were dense, and this factor attracted and favored the parasitic and predaceous taxa. A study of population densities substantiates this.

Table 2.—Total number of herbivorous, parasitic, and predaceous taxa recorded in the experimental communities.

EXPERIMENTAL COMMUNITY	NUMBER OF TAXA			
	Herbivores	Parasites	Predators	Total
Control	23	13	14	50
DDT	20	11	14	45
Rotenone	18	10	12	40
Parathion	19	2	3	24

Table 3.—Comparison of density in control plots recorded in 1957 and 1958.

TAXON	AVERAGE PER WEEK	
	1957	1958
Myzus persicae	21.5	21.4
Aphids	141.2	34.5
Lepidoptera	12.8	16.3
Flea beetles	1,107.1	1,007.9
Herbivores	643.5	355.9
Parasites	28.9	14.1
Predators	8.9	5.1

Table 4.—Mean monthly temperature (° F.) and rainfall (inches) for the experimental periods in 1957 and 1958.

YEAR	MONTHS				
	May	June	July	August	September
	Temperatures				
1957	53.9	67.4	67.4	63.6	60.0
1958	52.4	59.2	68.1	66.6	59.2
	Rainfall				
1958	3.30	3.09	5.55	1.43	3.15
1958	3.37	5.94	5.23	4.83	4.17

A total of 50 taxa was observed in the control community in 1957 and a like number in 1958. A total of 81% of these taxa was the same for both seasons. The density results for the control community presented in table 3 are comparable for both seasons. The green peach aphid, *Myzus persicae* (Sulz.), was separated from the aphid group to illustrate the agreement between seasons in spite of the change to all collards in 1958 and of significant changes in weather conditions that year (table 4). The greatest change occurred in populations of the cabbage aphid, *Brevicoryne brassicae* (L.) and this change is the principal reason for the average of 141.2 recorded in 1957 and an average of 34.5 recorded in 1958 for the aphid group. The data given in table 3 were obtained during two seasons which had varied weather conditions.

In the experimental communities, when insecticides were applied, significant changes in the density of the taxa occurred. For the sake of brevity, density trends of various groups of taxa are discussed together instead of

Table 5.—The average taxa density per week recorded in the four experimental communities.

Taxon	Control	DDT	Rotenone	Parathion
Aphids	162.7	1,106.3	1,481.2	20.3
Lepidoptera	12.0	4.4	0.9	1.0
Flea beetles	1,107.1	3.5	5.3	1.5
Herbivores	643.5	3.4	2.0	1.0
Parasites	27.8	481.7	621.5	0.4
Predators	8.9	7.6	3.2	0.7

Table 6.—The ratio of parasites to hosts and of predators to aphids in the various experimental communities.

Taxon	Experimental Community			
	Control	DDT	Rotenone	Parathion
		Parasites		
Aphids	9	34	30	1
Lepidoptera	57	22	22	8
		Predators		
Aphids	4.4	0.6	0.2	1.2

separately. With few exceptions, the trends are the same whether they are considered separately or are grouped (data filed in Comstock Memorial Library). The number of individuals for any taxon in each experimental plot varied, especially at the start of the season. Since all communities were initiated from immigrants, there were differences in time of establishment. To account for these initial differences, sampling was carried on during the entire season. Aphids, especially the cabbage aphid, were abundant in the DDT and rotenone communities (table 5 and fig. 1). Hervey (1946) also found cabbage aphid densities greater in DDT-treated plots than in control plots. The DDT and rotenone insecticides destroyed a sufficient number of predators to allow the aphids to reach outbreak density levels. Note the low predator densities in tables 5 and 6. Because these insecticides destroyed more predators than aphids, the aphids were allowed to increase. In the case of the parathion community, adequate control of both aphids and predators occurred (table 5).

All the insecticides listed were effective in reducing the lepidopteran populations (table 5 and fig. 2). In this respect, DDT was the least effective of the insecticides. The highest rates of parasitism of the lepidopterans were found in the control community (57%) and the lowest (8%) in the parathion community (table 6). A reduction in parasitic fauna usually follows a reduction in density of hosts. The reduction in parasite number, however, was much greater in the parathion community than in the rotenone community. Both communities had about the same density of lepidopterans, but the rotenone community had 22% of the lepidopterans parasitized while the parathion community had only 8% parasitized (tables 5 and 6).

Flea beetles were adequately controlled by all three insecticide treatments in the DDT, rotenone, and parathion communities (table 5 and fig. 3). The herbivores, other than aphids, lepidopterans, and flea beetles, were

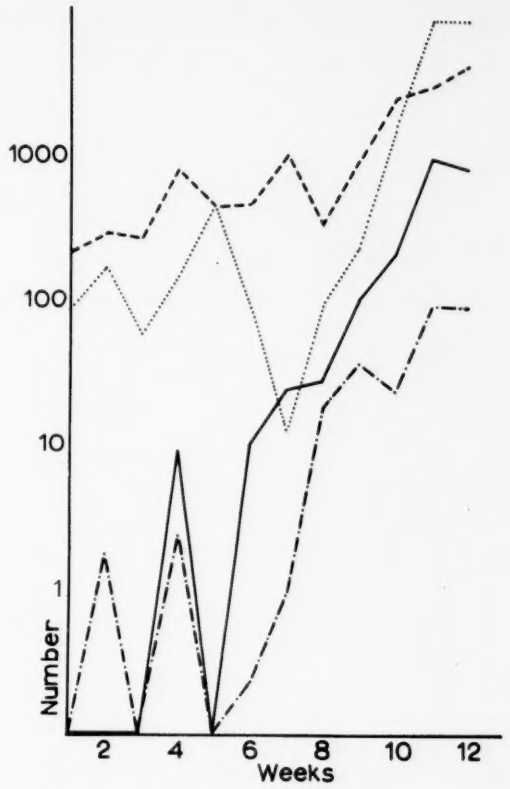

Fig. 1.—The log number of aphids per 20,000 square inches of plant area in the control (———), DDT (– – –), rotenone (····), and parathion (–·–·–) communities.

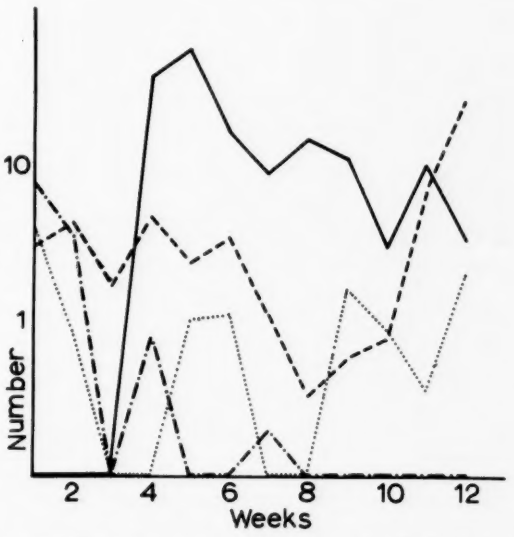

Fig. 2.—The log number of lepidopterans per 20,000 square inches of plant area in the control (———), DDT (– – –), rotenone (····), and parathion (–·–·–) communities.

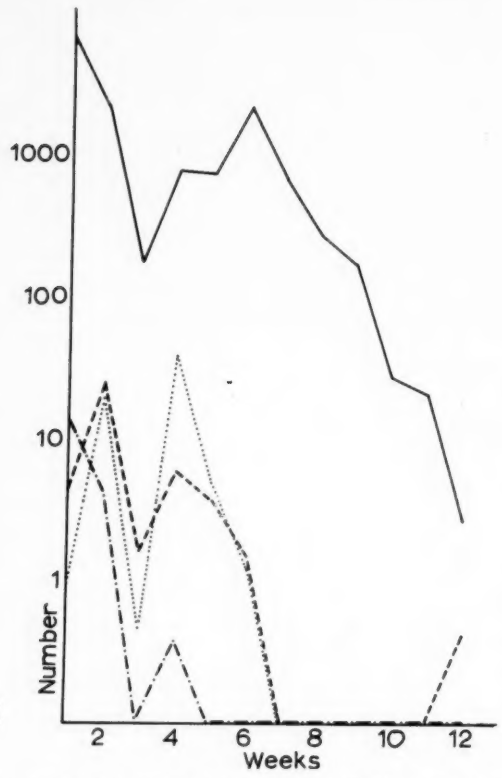

FIG. 3.—The log number of flea beetles per 20,000 square inches of plant area in the control (———), DDT (– – –), rotenone (· · · ·), and parathion (– · – · –) communities.

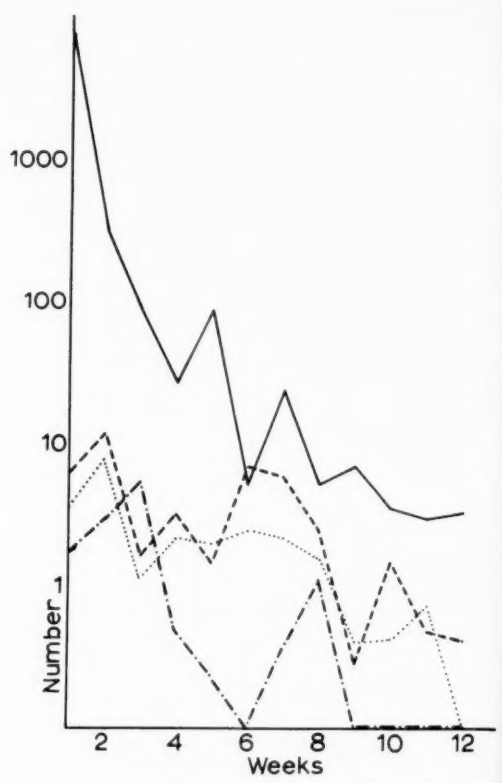

FIG. 4.—The log number of herbivores other than aphids, lepidopterans, and flea beetles per 20,000 square inches of plant area in the control (———), DDT (– – –), rotenone (· · · ·), and parathion (– · – · –) communities.

also controlled in the three insecticide-treated communities (table 5 and fig. 4).

Parasite densities were highest in the DDT and rotenone communities and lowest in the parathion community (table 5 and fig. 5). The high parasite density in the DDT and rotenone communities was caused by the high cabbage aphid density existing in these plots. Predators were most abundant in the control community, followed in abundance by the DDT, rotenone, and parathion community (table 5 and fig. 6). All insecticides were effective in suppressing the predator populations (table 6). Such reduction allowed the aphids to increase because predation pressure on the aphid populations was reduced. Subsequently, the dense aphid populations provided ideal conditions for the parasites which reached high density levels. Predators, however, were not absent from the DDT and rotenone communities even though large numbers were killed by the insecticides. The large aphid population served to attract predators to these communities.

DISCUSSION.—When insecticide control is directed at a single species without consideration of the ecology of the existing community, serious problems result. Following such a treatment some species are eradicated, others decrease in number, whereas some actually increase in number. This fact was substantiated by the results of this study. Species became extinct in the *Brassica oleracea* community because of insecticide treatments. Natural enemies were significantly reduced by the powerful insecticides, parathion, endrin, and malathion. In some instances when the natural enemies were destroyed, phytophagous species increased to outbreak levels.

The reason for this is that each species member of the community reacts differently to an insecticide than every other species member. For example, about 1.8 pounds of DDT per acre destroy 96% of the cabbage looper (*Trichoplusia ni* (Hbn.)), 85% of the cabbage butterfly (*Pieris rapae* (L.)), 75% of the diamondback moth (*Plutella maculipennis* (Curt.)), and 0% of the cabbage aphid (Smith & Harrison 1944, Hervey 1946). Because each species of the community is affected differently, the entire organization of the community changes. This is well substantiated in the *B. oleracea* community where numerous biotic factors influence the dynamics of the diamondback moth population. The diamondback moth is heavily parasitized by three hymenopteran parasites (data on file in Comstock Memorial Library). Various predators attack the moth. The attacks of these predators, notably the coccinellids, depends upon the numbers of three species of aphids (*Brevicoryne brassicae*, *Myzus*

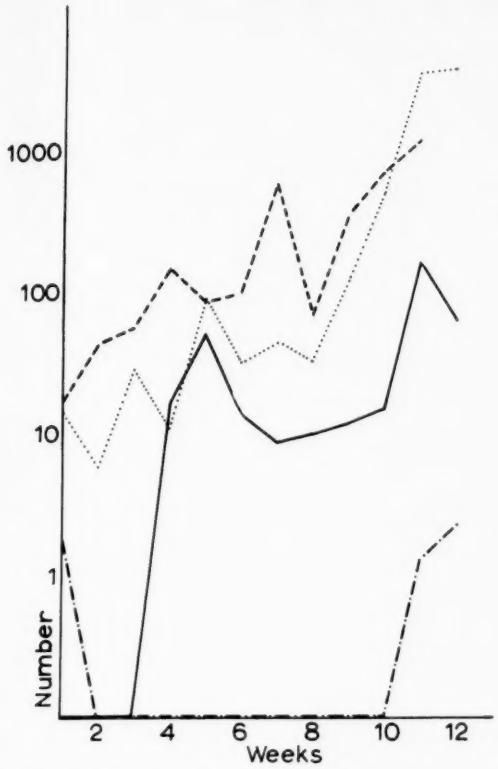

FIG. 5.—The log number of parasites per 20,000 square inches of plant area in the control (———), DDT (– – – –), rotenone (· · · ·), and parathion (– · – · –) communities.

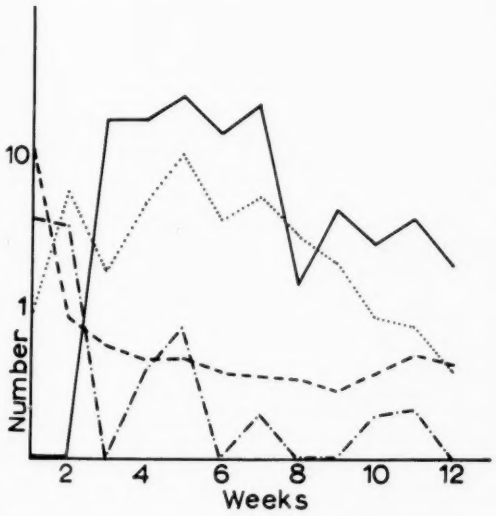

FIG. 6.—The log number of predators per 20,000 square inches of plant area in the control (———), DDT (– – – –), rotenone (· · · ·), and parathion (– · – · –) communities.

persicae, and *Rhopalosiphum pseudobrassicae* (Davis)) present. These species of aphids are greatly reduced by four species of parasitic Hymenoptera (Pimentel 1961). The populations of many species of herbivores interfere to a limited extent with the activities of the diamondback moth population. Hence, a change in any link of this network of the community organization will produce a change in the diamondback moth population. Because insecticides affect each species differently and because species are interdependent, it is impossible to predict exactly what insect control problems will result from an insecticide treatment until it is actually tested.

Certainly it is an economic necessity to regulate the density of some insect populations. However, thorough ecological study of both the individual species and the community as a whole is a necessary part of any insecticide program. Specific control agents, like systemic and microbial insecticides, are more advantageous than general insecticides, for they reduce the density of a specific pest species in the community. For example, the cabbage looper in the *B. oleracea* community can be effectively controlled with the nuclear polyhedrosis virus (*Borrelinavirus* sp.) without killing any other species (R. P. Jaques and D. Pimentel, unpublished data). Care is necessary when specific control agents are applied, because, as mentioned above, the destruction of any species link of the community network will produce some kind of a change in the community organization. General poisons are satisfactory only if they adversely affect the pest species more than the parasites, predators, and microorganisms and produce a desirable change in the community. Actual testing of various insecticides and the dosages to determine which can be used selectively without seriously upsetting the balance of the community in question is necessary. The control of the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)) demonstrates this approach (Stern *et al.* 1959).

Insecticides which "sterilize" the fauna of the community should be avoided because serious outbreaks may result when such insecticide pressure is eventually withdrawn. In the parathion community only five of a possible 27 parasitic and predaceous taxa remained, whereas a total of 19 of the possible 23 herbivorous taxa remained. Because the controlling parasitic and predaceous taxa had been destroyed, all of these herbivorous populations were capable of explosive multiplication whenever the insecticides were withdrawn. Permanent communities like forests are most susceptible to this kind of malpractice. Proper utilization of natural controlling forces such as predators and parasites affords a sound way to reduce the need for maintaining high insecticide pressure against pest species. Lower insecticide dosages would also reduce the often harmful insecticide accumulation in the soil which is known to destroy beneficial soil organisms (Menhinick 1960). Investigation of the natural controlling factors might eventually make possible control of the pest through some manipulation of the biotic and physical environment. Insect control without the usual complications of outbreaks, resistance, and residues is possible only when the ecology of populations and communities is thoroughly understood.

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Field Studies on the Control of Resistant House Flies[1]

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ABSTRACT

In 1959, Bayer Compound 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate), ronnel, and Dimetilan® (3-methyl-5-pyrazolyl dimethylcarbamate) were evaluated against resistant house fly populations in dairy barns and poultry houses. Bayer 29493 as a residual treatment, Dimetilan-impregnated fly bands, and ronnel-impregnated cords gave the most promising results.

Effective control was obtained with Bayer 29493 applied at the rate of 50 milligrams per square foot. As a bait treatment, this compound did not give satisfactory control except in one instance.

Dimetilan-impregnated fly bands, when installed at one dairy at the rate of 30 linear feet per 100 square feet of floor space, produced adequate control for more than 14 weeks. However, when installed at a second dairy at the rate of 6 linear feet per 100 square feet, satisfactory control was not obtained until 7 weeks after installation, after which fly counts remained below 21 for the balance of the season.

The ronnel-impregnated cords ($\frac{3}{32}$-inch diameter) gave effective control within 2 to 4 weeks after installation in June and July, and this control persisted for the remainder of the season.

House fly (*Musca domestica* L.) investigations in the Savannah, Georgia, area in 1959 stressed the evaluation of several organophosphorus compounds as residual-spray treatments, as baits, and as impregnated cords, against resistant fly populations.

MATERIALS AND METHODS.—Residual treatments were applied to all potential house fly resting surfaces in and around the test dairies at 100 p.s.i., using a power sprayer equipped with flat spray nozzles. The insecticides tested were Bayer Compound 29493[2] (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate) as a suspension or an emulsion applied at the rate of 50 milligrams per square foot, or as an emulsion-sugar formulation applied at the rate of 50:125 milligrams per square foot. Malathion (SF 59)[3] and Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate)[4] were applied as emulsions with sugar at dosages of 200:500 milligrams of toxicant-sugar per square foot. Commercially prepared wettable powders and emulsifiable concentrates were used to formulate the finished sprays.[5]

Cotton cord ($\frac{3}{16}$- or $\frac{3}{32}$-inch diameter) was treated with a 25% ronnel-xylene[6] solution (Kilpatrick & Schoof 1959a) and then installed as vertically suspended lengths (18-inch) from the ceilings or rafters, or from treated horizontal lines at a dosage of 30 linear feet of cord per 100 square feet of floor area. Commercially impregnated plastic fly bands which contained 2 grams of Dimetilan®[7] (3-methyl-5-pyrazolyl dimethyl carbamate) per meter were suspended horizontally from the ceilings or rafters. The bands, each 6.5 feet long, were installed at rates of 6.5 (as recommended by the manufacturer) or 30 linear feet per 100 square feet.

Bait tests were made with Bayer Compound 29493 as a 0.1% emulsion and as a 0.5% dry bait, and with Dimetilan as a 1.0% dry bait. The emulsion bait, which contained 12.5% sugar, was applied utilizing 1-gallon chicken-watering devices (6 quarts of bait per 1,000 square feet) (Kilpatrick & Schoof, 1959b); or by spraying the bait, at weekly intervals, on three thicknesses of cellucotton stapled to 16- by 16-inch plywood boards. The latter bait stations, sprayed with approximately 1 quart of the emulsion bait per station, were installed at the rate of three stations per 1,000 square feet of floor area. The dry bait was prepared by spraying 22.7 grams of technical material (dissolved in enough acetone to insure thorough mixing) on a mixture of 4,063 grams of corn grits and 454 grams of sugar. It was distributed in bait stations (18- by 18-inch plywood trays) at a rate of 4 ounces of the bait per 1,000 square feet. The same application technique was used with the Dimetilan 1.0% dry bait (commercially prepared) which was applied at two rates: 2 ounces of bait per 1,000 square feet in two treatments a week apart and 4 ounces of bait per 1,000 square feet in three treatments 3 weeks apart.

All treatments were evaluated by weekly grill counts of

[1] From the Technical Development Laboratories, Technology Branch, Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia. Accepted for publication August 1, 1960.

[2] Supplied by Vero Beach Laboratories, Vero Beach, Florida.

[3] Supplied by American Cyanamid Company, Stamford, Connecticut.

[4] Supplied by California Spray-Chemical Corporation, Moorestown, N.J.

[5] Use of trade names in this article is for identification only and does not constitute endorsement by the Public Health Service.

[6] Supplied by The Dow Chemical Company, Midland, Michigan.

[7] Supplied by Geigy Chemical Corporation, Ardsley, New York.

Table 1.—House fly control in five dairy barns near Savannah, Georgia with Bayer 29493 residual sprays, 1959.

Weeks	Fly Counts—3-Week Moving Average				
	Dairy "Be" 50 Mg./Sq. Ft. (S)[a]	Dairy "Ba" 50 mg./Sq. Ft. (E)[b]	Dairy "Ra" 50 mg./Sq. Ft. (E)	Dairy "C" 50:125 Mg./Sq. Ft. Toxicant-Sugar (E)	Dairy "W" 50:125 Mg./Sq. Ft. Toxicant-Sugar (E)
Before Treatment					
3	100	74	64	112	36
2	102	186	176	116	87
1	138	302	292	127	109
0	154	321	255	149	106
After Treatment					
1	2	8	3	6	5
2	3	13	5	4	5
3	3	22	6	5	9
4	2	24	12	8	18
5	2	24	16	8	21
6	3	20	16	6	15
7	3	12	37	4[c]	13
8	4	11	45		16
9	6	15			12
10	9	23			7
11	9	36			11
12	14	46			20
13	34	65			20[d]
Date treated	July 20	May 28	July 17	June 26	June 2

[a] S = suspension.
[b] E = emulsion.
[c] Herd moved August 12, 1959.
[d] Indices below 20 through 23rd week.

Table 2.—House fly control in two dairy barns near Savannah, Georgia, with Dimetilan fly bands, 1959.

Weeks	Fly Counts—3-Week Moving Average	
	Dairy "Mo" 6.5′/100 Sq. Ft.	Dairy "Z" 30′/100 Sq. Ft.
Before Treatment		
3	20	120
2	128	132
1	292	126
0	362	133
After Treatment		
1	53	14
2	52	13
3	50	15
4	46	17
5	57	16
6	51	13
7	39	13
8	16	15
9	18	16
10	16	12
11	20	7
12	21	4
13	17	8
14	18	14
15	20	16
16	15	14
17	12	10
18	14	5
19	14	2
20	14	2
21	14	
22	15	
23	10	
24	7	
25	4	
26	3	
Date treated	May 23	June 25

the house flies at each dairy or chicken farm. Five counts were made at each of four general types of locations (entrance, barn floor, stanchions, and feed room) known to be highly attractive to *M. domestica*. The weekly index was the average of the four highest counts, one from each type location. Indices of 10 or below were considered as excellent control; those between 10 and 20 as satisfactory control.

RESULTS.—At the dairies treated with Bayer 29493 as a residual spray, fly indices dropped to levels of excellent control (table 1) immediately after application. The suspension (50 milligrams per square foot) provided excellent control for 11 weeks at dairy "Be" with satisfactory control for 1 additional week. The emulsion (50 milligrams per square foot) gave satisfactory to excellent control for 6 weeks at dairy "Ra." At dairy "Ba," the same treatment yielded 10 weeks of indices below 25, 6 weeks of which represented satisfactory control. The emulsion with sugar (50:125 milligrams per square foot) maintained satisfactory to excellent control at dairy "W" for 22 weeks, and excellent control at dairy "C" for 7 weeks, after which the dairy herd was moved, making further evaluation impossible.

The malathion-sugar emulsion (200:500 milligrams per square foot) resulted in only 1 week of control at dairy "Ra." The Dibrom-sugar emulsion (200:500 milligrams per square foot) gave 4 weeks of excellent to satisfactory control at dairy "Ba."

The emulsion baits with Bayer 29493 produced contradictory results. When offered in chicken-watering devices at dairy "H," satisfactory control was not obtained although the indices were reduced from ranges of 115 to 300 to levels of 50 to 70. This same formulation applied at weekly intervals to cellucotton pads at a poultry farm gave 2 weeks of reduced indices followed by 5 weeks of excellent control. The dry bait containing 0.5% of Bayer 29493, and applied at the rate of 4 ounces per 1,000 square feet, did not provide satisfactory control at the second poultry farm. At dairy "H," the dry bait of 1% Dimetilan (4 ounces per 1,000 square feet) gave only 1 week of satisfactory control; while at a poultry farm, control was not obtained when the bait was applied at the rate of 2 ounces per 1,000 square feet.

The Dimetilan-impregnated fly-bands at dairy "Mo" (6.5 feet per 100 square feet) produced 7 weeks of reduced but unsatisfactory indices followed by satisfactory control for the rest of the season (19 weeks). An installation of these bands at a rate of 30 feet per 100 square feet at dairy "Z" reduced fly indices to control levels immediately, and satisfactory to excellent control was maintained for the remainder (20 weeks) of the season (table 2). The ronnel-impregnated cords ($\frac{3}{32}$-inch diameter) at three dairies produced satisfactory to excellent control of fly populations for the entire season (table 3). In contrast, cords ($\frac{3}{16}$-inch diameter) at dairy "Rg" yielded 14 weeks of reduced but unsatisfactory indices followed by excellent control for the remaining 8 weeks.

DISCUSSION.—The insecticide Bayer 29493 offers considerable promise as a residual treatment for the control of resistant house fly populations. The control achieved was equal to or better than that previously obtained with

Table 3.—House fly control in four dairy barns near Savannah, Georgia, with ronnel-impregnated cords, 1959.

	Fly Count—3-Week Moving Average			
	$\frac{3}{16}$" Diameter	$\frac{3}{32}$" Diameter		
Weeks	Dairy "Rg"	Dairy "Br"	Dairy "Km"	Dairy "T"
	Before Treatment			
3	443	62	526	222
2	409	63	872	322
1	345	50	852	280
0	345	54	644	183
	After Treatment			
1	113	3	37	16
2	107	3	18	51
3	66	3	6	81
4	50	2	7	54
5	64	2	8	20
6	51	2	8	12
7	29	2	6	16
8	20	2	8	18
9	22	2	6	19
10	29	2	6	12
11	27	2	5	6
12	22	2	6	6
13	24	4	9	6
14	21	8	11	6
15	12	9	12	6
16	10	7	12	6
17	10	4	13	5
18	9	2	12	4
19	7		9	4
20	7		9	4
21	6		10	3
22	4		9	2
Date treated	June 15	July 9	June 12	July 10

other organophosphorus insecticidal sprays in the Savannah, Georgia, area. The suspension formulation was slightly more effective than that of the emulsion alone. The addition of sugar to the emulsion formulation appeared to increase the residual effectiveness of the formulation, but further tests are required to substantiate this point. As a bait treatment, the compound produced reduced fly indices, but failed to lower the counts to control levels except in one instance.

When installed at the same rate, the Dimetilan-impregnated fly-bands were about as effective as the ronnel-impregnated cords ($\frac{3}{32}$-inch diameter), giving satisfactory control within a week after installation at the single dairy treated. Although lower counts were obtained with the ronnel cords at two dairies ("Ba" and "Km"), at a third dairy ("T") the counts were higher than those obtained with the Dimetilan fly-bands (dairy "Z"). At this dairy ("T"), the cords in the calf barn became entangled by spider webs, and a sudden increase in the fly population occurred. However, when the webs were removed, the fly indices returned to satisfactory levels. Similar difficulties were experienced with the $\frac{3}{16}$-inch diameter cords at dairy "Rg," where counts were reduced from the 300 to 400 range to the 25 to 60 range without control levels being obtained until the 15th week.

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BOOK REVIEW

Modern Insecticides and World Food Production, by F. A. Gunther and L. R. Jeppson. xv+284 pp. incl., 57 illus. John Wiley and Sons, Inc., New York 1960. Price $8.50.

This book is not addressed to specialists in entomology or food production but is designed to bring out the relationship between the two and to show the need for insect control. It starts with the basic biology of insects and traces man's efforts to control insects that compete with him for the food supply. The formulation and application of insecticides and the studies of the resulting deposits and residues are discussed in relation to the Federal laws that pertain. This is followed by a discussion of individual insecticides that are in common use. The Appendix lists the approximate mammalian toxicities of these materials. In general this book should serve as a convenient source of factual material for the writers of popular articles on insecticides, and one can but wish that the writers of the "scare" articles would consult this work before penning their alarming but unfactual manuscripts. Students in agriculture should find this book most helpful in their understanding of the place of insecticides in modern food production.

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Lygus Bug Damage to Table Beet Seed Plants[1]

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ABSTRACT

The possible effect of lygus bug infestations on quantity and quality of developing table beet seeds was investigated by artificially infesting caged portions of plants in the field, each portion being approximately one-third of one plant. The caged parts of bolting plants were exposed to constant numbers of male lygus bugs, *Lygus hesperus* Knight, with levels of infestation continuously maintained at 1, 2, 4, 8, and 16 bugs per cage for periods of 40 and 42 days.

Yield data from two experiments show that average amounts of seed per unit of plant exposed to lygus bugs ranged from 15% to 25% below the yields recovered from control plants. The differences in yields between the portions of infested plants and the controls or between the several levels of infestation were not significant. However, when the yield data from the second of two experiments were corrected for inequalities between caged portions of plants, and computed on a per plant basis, differences between the checks and the several groups of exposed plants appear to be significant. The converted data show that equivalents of 7.5 to 50.7 bugs per plant (or 2 to 16 bugs per cage) reduced seed yield 33% to 44%. The highest level of infestation, 16 bugs per cage, also perceptibly increased the yield of seeds classified as small seeds.

Exposure to lygus bugs caused a decrease in the weight of individual seeds of two size categories, those graded as large seeds and as small seeds. The reduction in weight of seeds appeared to be economically important at levels of 8 to 16 bugs per cage (22.8 to 50.7 bugs per plant).

The numbers of germinating beet seeds and viable germs per seed were substantially reduced by exposure to lygus bugs in two experiments, decreasing as the number of bugs were increased, and were significantly lower for both the large and small seeds. The decrease in percentage of beet seed viability approached 60% at 8 or 16 bugs per cage. A minimum germination of 85% is needed to produce an adequate number of plants in the field.

Injury by lygus bugs to seed crops has been reported for alfalfa, beans, other legumes, carrots and other umbelliferous seed plants, and sugar beets. The kind of damage caused by these bugs may be plant deformation, die-back, and reductions in seed yield and seed viability. The degree of damage also varies considerably, depending on the type of damage, the crop involved, and the numbers of bugs present.

Investigations on sugar beet plants by Hills (1941, 1943) showed that several species of Lygus (*L. oblineatus* Say, *L. hesperus* Knight, and *L. elisus* van Duzee) were responsible for the production of nonviable sugar beet seed. Hills & Taylor (1948, 1950) further demonstrated that the greatest reduction in seed viability and yields resulted from the feeding of the bugs on the soft, newly formed seeds of sugar beets.

The investigations on lygus bugs reported in this paper were concerned with the kind and degree of damage sustained by table beets grown for seed at Davis, California. The degree of damage was studied by maintaining definite numbers of bugs in field cages covering portions of the plants.

GENERAL PROCEDURE.—Approximately one-third of the spikes and spikelets of each test plant were enclosed in a cheesecloth cage, 9 inches in diameter by 24 inches high. Only young bolting plants were so caged. All enclosed plant parts were sprayed with a 0.015% dilution of a commercial pyrethrum and rotenone preparation, in order to eliminate the unwanted insects. Plants destined for exposure to lygus bugs were sprayed three times after installation of cages and before the introduction of insects. The caged check or control plants were sprayed periodically for the duration of the test period.

Cages of moderate size placed over substantial portions of each test plant were judged to be the most practical for the purpose of these tests. Larger cages, covering whole plants, were not used because of complications in accounting for exact numbers of insects. Small cages, to enclose individual shoots or spikelets, could not be placed during the early bolting period of the plants (and later placement would have assured the presence of unwanted insects). They would also have introduced additional difficulties in evaluating proportional parts of whole plants.

In the first experiment (1958), males of *Lygus hesperus* were introduced into the cages when the plants were beginning to bloom (15% bloom). The use of males only precluded the build-up of populations of immature stages and effects of oviposition. The tests involved continuous exposure of test plants to concentrations of 1, 2, 4, and 8 bugs per cage, and nine plant replicates of every concentration, including the controls. All of the infested plants were inspected every other day for dead bugs and the dead ones were replaced with live ones. The test period was continued for 40 days. The beets were then allowed to mature further for an additional week. The caged parts were then cut (but left in the cages) and air-dried for threshing and cleaning of the seed.

The general design of the first experiment was incorporated in a second experiment completed during 1959. The plants were caged as before but the exposure to lygus bugs was started just before the blooming period of the plants. The tests comprised exposures to concentrations of 2, 4, 8, and 16 male bugs per cage for 42 days. The plants were then allowed an additional maturation period of 10 days before cutting. There were seven one-plant replications per concentration.

Seeds were recovered from exposed parts of each plant by opening the cage and manually threshing the dry spikelets in a tub. Each lot of seed was cleaned twice in a Clipper office cleaner and weighed to hundredths of a gram. In the second experiment the cleaner was adjusted

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[2] The author gratefully acknowledges the assistance of Nurseryman, John Campbell, University of California, Davis.

Table 1.—Average values obtained for yield, weight per 100 seeds, and viability of table beet seeds, according to numbers of male lygus bugs per cage, Davis, California, 1958 and 1959.[a]

CATEGORY	NUMBERS OF INSECTS PER CAGE					
	0	1	2	4	8	16
	First experiment, 1958					
Seed yield (gm.)	14.5a	11.1a	12.3a	11.3a	10.8a	—
Yield reduction (%)	—	23	15	22	25	—
Weight of 100 seeds (gm.)	1.45d	1.28c	1.26bc	1.12ab[b]	1.11a[b]	—
No. of seeds per avdp. ounce	1,958	2,219	2,253	2,535	2,558	—
No. of viable seeds in samples of 100 seeds	83.2b	79.2ab	77.2ab	66.9a	70.1ab	—
No. of viable germs per 100 seeds	102.0b	91.9ab	94.3ab	62.6a[b]	69.9a[b]	—
	Second experiment, 1959					
Seed yield (gm.)						
Large seeds	29.9a	—	23.2a	22.3a	21.9a	24.5a
Small seeds	0.6a	—	0.5a	0.7a	0.9a	1.4a
Yield reduction (%)	—	—	22	25	25	15
Weight of 100 seeds (gm.)						
Large seeds	1.52bc	—	1.54c	1.35ab	1.34ab	1.32a
Small seeds	0.34b	—	0.28ab	0.29ab	0.29ab	0.26a[b]
No. of seeds per avdp. ounce						
Large seeds	1,862	—	1,838	2,094	2,110	2,142
No. of viable seeds						
100 large seeds	85.1b	—	74.3ab	73.3ab	64.1a[b]	61.7a[b]
100 small seeds	22.4b	—	8.8a	12.0ab	9.8ab	4.0a[b]
No. of viable germs per 100 large seeds	127.5b	—	108.7ab	96.8ab	80.8a	74.2a[b]

[a] Significant differences shown in this and in table 2 were computed according to Duncan's Multiple Range Test (1955). Significant differences between means (5%) are indicated when compared values have no letters in common.
[b] These values are significantly different from the checks at the 1% level.

to separate large from small seeds.

EFFECT OF LYGUS BUGS ON SEED YIELD.—The data on yield of table beet seed are presented in table 1 as averages for one-third (caged) portions of the test plants. The average yield of seed in grams (per cage basis) did not vary significantly between controls and infested plants. Although all of the seed yields from infested plants were 15% to 25% less than those from the uninfested lots in both experiments, there was no evident correlation between numbers of bugs and yield of seed. The highest level of infestation did cause an increase in the production of small seeds, but this increase too was not significant.

Variations between the plant units selected for caging probably introduced sufficient error in yield estimates to confuse the causal relationship between numbers of insects and seed yield. The seed yield from the fractional part of each plant actually enclosed in a cage was equated to total plant yield with greater precision in the second experiment. The numbers of secondary and tertiary spikelets in enclosed and unenclosed parts of each test plant were determined. This ratio, or conversion factor, provided a workable basis for transforming yield per part plant to yield per plant. The derived values obtained in the second experiment are given in table 2 and indicate that 16 bugs per cage (the top range of exposure) provided an average density of 50.7 bugs per plant, a substantial level of infestation. Conversion of the data increased to significance the yield differences (for large seeds) between control and exposed plants, but did not provide data that indicated any change in the relationship between numbers of lygus bugs and seed yields.

The seed losses and blasting sustained by the table beet seed plants in consequence of lygus bug attack are known to be considerably less than for other vegetable seed plants, such as carrots (Carlson 1956). Hills & Taylor (1950) also found that rather high concentrations of lygus bugs are required to reduce yields of sugar beet seed. The smaller losses of table beet seed seem to be related, at least in part, to the tight adherence of the seeds to the stems, from which they are not easily dislodged. There is, therefore, less drop of damaged seeds.

EFFECT OF LYGUS BUGS ON WEIGHT OF SEEDS.—The weight of 100 seeds was significantly greater for the lots obtained from uninfested plants than for the lots obtained from all the infested plants of the first experiment (table 1). These weights were obtained as averages for 100 seeds, randomly selected, from each replicate. The effect of increased numbers of bugs was evident in reduced seed weight when large and small seeds were not segregated. This decrease in seed weight was very significant at a concentration of eight bugs per cage.

A similar trend was demonstrated in the second experiment. In this case, however, the seeds were partitioned

Table 2.—Average seed yield per plant obtained for different numbers of lygus bugs per caged portions of table beet seed plants, Davis, California, 1959.

CATEGORY	NUMBER OF BUGS INTRODUCED PER CAGE				
	0	2	4	8	16
	Calculated Number of Bugs per Plant				
	0.0	7.5	12.7	22.8	50.7
Seed yield (gm.)					
Large seeds	116.4b	74.2a	65.2a[a]	68.2a[a]	78.1a
Small seeds	2.5a	2.0a	2.1a	2.6a	5.1a
Yield reduction (%)					
Large seeds	—	36	44	41	33

[a] Significantly different from the checks at the 1% level.

into two size categories during the cleaning process. Larger numbers of bugs (16 per cage) were required to produce a significant reduction in the weight of 100 of the large seeds. The control plants also produced significantly heavier seeds of the small size than the plants exposed to the highest concentration of insects (16 bugs per cage). Otherwise, variations in levels of infestation did not cause meaningful differences in their weights.

Decreases in unit seed weights also indicate that lygus feeding affects the size of seeds, because the number of seeds required per avoirdupois ounce is increased. This decrease in weight of seeds became economically meaningful when the number of bugs was maintained at 8 and 16 per cage, an infestation equivalent to 22.8 and 50.7 bugs per plant, respectively.

EFFECT OF LYGUS BUGS ON SEED VIABILITY.—The 100-seed samples selected for determinations of the weight of seed were utilized in germination tests. Viability was tested by distributing each sample of seed in a plastic sandwich box containing a blotter soaked with distilled water. Once germination started, the number of viable seeds and of viable germs per seed were counted daily until the counts leveled off.

In the first experiment approximately 83% of the seeds from control plants produced at least one viable germ (table 1). This value was reduced to about 70% for the highest level of bug infestation. The four infested groups of plants were not distinguishable with respect to seed viability, and in only one of the tests (4 bugs per cage) was seed viability reduced significantly below the value observed for the check plants. However, the number of viable germs per seed was significantly reduced by either four or eight bugs per cage.

In the second experiment, the numbers of viable seeds of the large category were substantially reduced by exposure to lygus bugs. Decreases in viability, below that obtained in the check, were significant for exposure levels of 8 and 16 bugs per cage. The number of viable germs per 100 large seeds also declined as the numbers of bugs were increased. Here, too, the values obtained for the two highest levels of infestation were significantly lower than the values observed for the control samples. Maximum loss of viable germs (possible one to five per seed) amounted to 42% per 100 seeds. The viability of small seeds from plants exposed to 16 bugs per cage dropped 82% below the check samples, but nevertheless the loss in viability among the small seeds was erratic within the range of exposure levels.

The decrease in viability of the large seeds at 8 or 16 bugs per cage was a very important one. This is because seeds having a percentage germination only slightly more than 60% are not used commercially. The accepted minimum viability of beet seeds is 85%, as obtained from the check samples. Other conditions being favorable, field plants may produce seeds of 90% to 95% viability when lygus bugs are properly controlled. The data indicate that an economical decrease in viability occurred when the bugs were maintained at infestation levels calculated to be 22.8 to 50.7 per plant. Levels of four bugs per cage (12.7 bugs per plant) may be sufficient to cause a significant reduction in seed viability.

DISCUSSION.—Populations of lygus bugs in table beet seed fields are often sampled with an insect sweep net. It is estimated that one sweep of a standard net effectively samples approximately one-sixth of one to two plants. The data presented on numbers of lygus bugs in relation to weight of seeds and seed viability indicate that two to four bugs per sweep constitute a potentially damaging infestation.

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Insecticidal Susceptibility of *Nysius raphanus*, a Pest of Cotton[1]

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ABSTRACT

An outbreak of *Nysius raphanus* Howard occurred in the San Joaquin Valley of California during the spring of 1959 and 1960. Population build-up occurred on *Sisymbrium irio* L., *Capsella Bursa-pastoris* (L.) Medic. and *Lepidium nitidium* Nutt. Migration to cotton occurred in mid-May, with resultant destruction of seedling plants. In field experiments endrin and heptachlor gave satisfactory control while Sevin® (1-naphthyl methylcarbamate) was relatively ineffective. In laboratory experiments using a bioassay vial method of exposure, the approximate micrograms of several toxicants needed to give 50% mortality were: malathion 0.0017, dieldrin 0.3, endrin 1.0, Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) 1.6, toxaphene 30, DDT 80, and Sevin 50. Laboratory results with Sevin were erratic and inconclusive.

Members of the genus *Nysius* (Lygaeidae) have been recognized as occasional pests of cotton since 1906 (Sanderson 1906). Outbreaks are sporadic and usually restricted to a few areas in any particular year. A recent revision of this group by Barber (1947) indicates that two species are primarily involved, *N. raphanus* Howard and *N. californicus* Stål. The former species, referred to as *N. minutus* Uhler by Smith (1942) occurs on cotton in California. While the latter species also occurs in California, it has been reported as a pest of cotton only in Mexico (Rude 1950) where fruiting forms (squares and bolls) were attacked.

An outbreak of *N. raphanus* occurred on seedling cotton in several areas of the San Joaquin Valley during May of 1959 and 1960. It increased to epizootic proportions on preferred weed hosts belonging to the mustard family (*Sisymbrium irio* L., *Capsella Bursa-pastoris* (L) Medic., *Lepidium nitidium* Nutt. and others). When these hosts matured or were destroyed by cultivation, the adults and nymphs moved to adjacent rows of seedling cotton. Greatest destruction occurred in fields that were "skip-row" planted (a common practice in which alternate four-row areas remain unplanted or planted to small grains), and in cotton adjacent to weedy fields of flax, barley, or fallow.

Observations made during the late winter and spring of 1960 indicate that adults appear on *Sisymbrium irio* in late February and early March. Nymphs are detectable in late March and apparently a second spring generation develops by mid-May when movement to cotton occurs.

N. raphanus feed gregariously, 50 or more bugs attacking a single plant. Plant juices are withdrawn from stems, petioles and leaves and the plants soon assume a dried, scorched appearance, after which the bugs move to adjacent plants. Stems and leaves may become encrusted with feces. Injured plants and deposit of excrement on leaves and stems are illustrated in figure 1.

These insects remain hidden beneath clods or trash through the night and during the mid-day period when temperatures are high. They may be observed feeding on the plants during midmorning and late afternoon. Strong winds cause the bugs to seek cover. During periods of hiding, feeding may continue on the stems at and beneath the ground level.

EXPERIMENTAL PROCEDURES AND RESULTS.—*Field Experiments.*—Parathion and dieldrin have been recommended in California for control of this pest. Experimental data are meager and results obtained by growers have been highly variable with these materials.

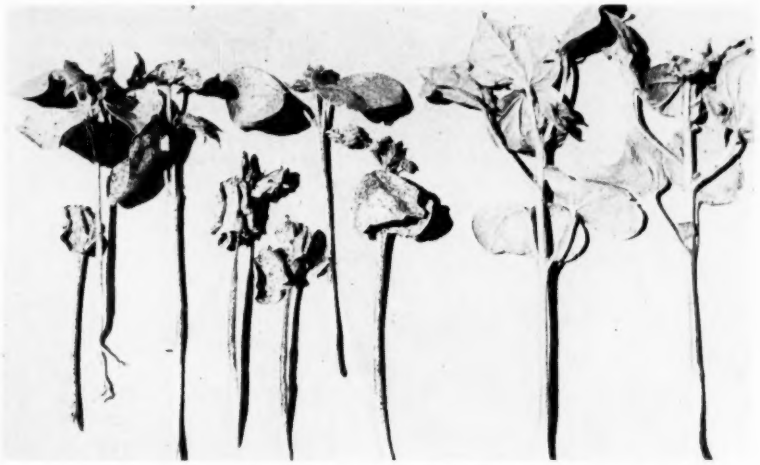

FIG. 1.—Plants showing degrees of severity of attack by *Nysius raphanus* Howard. Note excrement deposit as black specks on all plants. May, 1959.

[1] Accepted for publication August 1, 1960.

Table 1.—Results with endrin, heptachlor and Sevin applied as concentrated sprays on *Nysius raphanus*, May 6, 1959. Shafter, California.

MATERIAL AND ACTUAL TOXICANT/A. (LB.)	BUGS PER 4" CIRCLE (ADULTS AND NYMPHS)[a]			
	May 6	May 7	May 8	May 11
Endrin, 0.5	32.5	6.8	1.5	0.8
Heptachlor, 0.6	14.5	9.8	7.4	4.2
Sevin, 1.5	19.1	31.5	15.2	7.2

[a] Average of 15 counts (5 places in each of 3 replications). Counts on May 6 were made before treatments were applied.

An investigation was undertaken to determine the effectiveness of several compounds against this pest. Endrin, heptachlor and Sevin® (1-naphthyl methylcarbamate) were applied with a ground sprayer at 0.5, 0.6 and 1.5 pounds per acre, respectively, to skip-row cotton with each treatment replicated three times. Materials were applied at 20 gallons per acre through a single nozzle over each row, with an additional nozzle directed into weeds at each edge of the four-row panel.

Effectiveness was determined by observation and by pretreatment and posttreatment counts of bugs occurring in a 4-inch diameter circle inscribed on the ground after trash and clods were lightly raked aside. Counts were made at five places in each replication. Results are given in table 1. Endrin and heptachlor apparently gave satisfactory control with no further injury to the cotton evident. Sevin appeared to be ineffective. However, constant migration of bugs from untreated weed areas to treated areas made evaluation of the three materials difficult.

Laboratory Experiments.—To overcome the variability of field experiments, laboratory evaluation of pesticides was undertaken. In 1959 dieldrin, endrin, DDT, toxaphene, and Sevin were tested at several rates according to the method used by Andres *et al.* (1955) for lygus bugs. Technical-grade toxicant plus 0.5 lambda of mineral oil was dissolved in petroleum ether, then deposited onto the wall of a bioassay vial measuring 25 mm. in diameter and 45 mm. high. About 200 adult bugs were placed in each vial for 1 hour, with each test replicated 3 or 4 times. The bugs were then transferred and held 24 hours on green beans following which mortality was determined. The formula developed by Abbott (1925) was applied to determine the per cent mortality.

The micrograms of toxicant per vial necessary to achieve at least 97% mortality were: Endrin 1.0, dieldrin 2.0, toxaphene 80.0, and DDT 80.0. Sevin gave erratic results with at least 300 micrograms required to give 90% mortality.

Further tests were undertaken in 1960 using the same laboratory method, but omitting the oil in this series of experiments and limiting the number of bugs exposed in each replicate to 20. Replication was restricted by availability of *N. raphanus* to 3 to 5 replicates at the higher and lower dosages and 4 to 6 replicates in the LD_{40} to LD_{60} range. Malathion and Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) were included in addition to the five materials included previously.

The results for all compounds except Sevin are presented by probit-log dosage relationships in figure 2. Variability of the results made a probit analysis impossible and the lines were fitted by eye to the average mortality values.

The approximate quantity in micrograms needed to give an LD_{50} for the several compounds are malathion 0.0017, dieldrin 0.30, endrin 1.0, Dipterex 1.6, toxaphene 30, DDT 80 and Sevin about 50. The data for Sevin are not considered reliable as the results during both years were erratic. Endrin at 1 and DDT at 80 micrograms were found to give 97% control in 1959 and 50% control in 1960. The differences may be related to the use of oil in the 1959 formulations or to differences in susceptibility in the field population.

A limited number of tests were conducted with late-instar nymphs. While the data are inconclusive, this stage is apparently much less susceptible than the adult to all the above-mentioned materials.

DISCUSSION.—Malathion, the most effective compound tested in the laboratory, was two hundredfold more toxic than the next most effective compound, dieldrin. Dieldrin, endrin and Dipterex were about equally effective against this pest while toxaphene and DDT were relatively ineffective. In view of field and laboratory results with endrin, this compound and malathion, dieldrin and Dipterex should all prove to be effective in the field. Sevin is ineffective against this pest at moderate rates of application. Lack of control with dieldrin or similar mate-

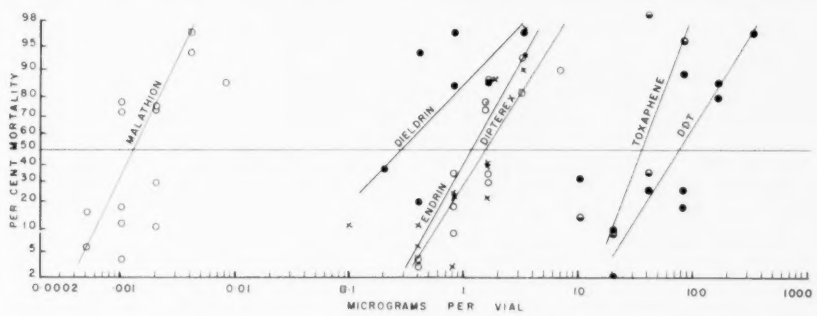

FIG. 2.—Relative effectiveness of six compounds on *Nysius raphanus* Howard, by bioassay vial method, May, 1960. Malathion and Dipterex, open circles; dieldrin and DDT, closed circles; endrin, X; and toxaphene, half-closed circles.

rials appears to be the result of improper timing with applications needed at a time when the bugs are exposed and feeding.

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Effect of Certain Antibiotics and Other Compounds on the Two-Spotted Spider Mite[1]

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ABSTRACT

Egg laying by the two-spotted spider mite (*Tetranychus telarius* (L.)) was strongly inhibited by cycloheximide (3-[2-(3,5-dimethyl-2-oxycyclohexyl)-2-hydroxyethyl] glutarimide), an antibiotic useful against powdery mildew and some other fungous diseases. This inhibition occurred on peach, pear, apple, and rose leaves. The mites were also suppressed on apple leaves when cycloheximide was combined with Sevin® (1-naphthyl methylcarbamate). This antibiotic is systemic, and mites on potted peach trees were controlled by 25 to 200 p.p.m. for 2 to 6 weeks, the period of protection increasing with the concentration. Another antibiotic, cytovirin, a material extremely hazardous to handle, was about equal to cycloheximide in suppressing egg laying, but several other antibiotics, including derivatives of cycloheximide, showed much less or no activity. Both cycloheximide and cytovirin are phytotoxic at low concentrations. Pear seedlings were much more susceptible than apple and peach plants which were not injured in greenhouse tests by levels effective for the control of mites.

In studies of possible effects of nutritional and hormone sprays, fungicides, and insecticides on orchard mites, it was found that egg laying by the two-spotted spider mite (*Tetranychus telarius* (L.)) was strongly inhibited by cycloheximide,[3] also known as Acti-dione, an antibiotic material that caused only a slight to moderate mortality of the adult. In some of the preliminary tests egg laying was reduced 95% to 99%, and single applications of 50, 100, and 200 p.p.m. on potted peach trees in the greenhouse gave excellent control on leaf disks cut from the sprayed trees for several weeks. These results and similar effects of another antibiotic, cytovirin, produced by an unidentified streptomycete and extremely hazardous to handle, have been reported briefly (Harries 1960).

Cycloheximide was discovered in 1946 by Ford & Leach (1948) in beers from streptomycin-producing strains of *Streptomyces griseus*. It was the first antibiotic reported to control a plant disease (Felber & Hamner 1948). Ford *et al.* (1958) reported on its agricultural uses and phytotoxicity. Studies of the mode of action of cycloheximide in yeasts have shown that it may inhibit both nucleic acid and protein synthesis (Kerridge 1958) or enzyme activity (Latuasan & Berends 1958).

Following discovery of the effect of cycloheximide on egg laying by the two-spotted spider mite on peach (Harries 1960) there was interest in its ability to exert a similar effect on other host plants, and to suppress mite increases following use of certain insecticides, such as Sevin,® and also in the possible effectiveness of derivatives of cycloheximide, other antibiotics, antiviral and anticancer compounds, and some other materials. The results of laboratory studies early in 1960 at Wenatchee, Washington, are presented herein.

The results reported by Harries (1960) and those reported herein, unless otherwise indicated, were obtained by using a leaf-disk technique, as follows:

MATERIALS AND METHODS.—Plants grown in 4-inch pots in the greenhouse were sprayed with a specially constructed atomizer while being rotated on a turntable set up in a chemistry hood as a safety means. The atomizer was rigidly mounted about 20 inches from the plant, and the sprays were stirred during the application. Distilled water was used in mixing the sprays, and one drop of X-77, a surfactant consisting of alkylaryl-polyoxyethylene glycols with free fatty acids and isopropanol, per 100 ml. was added to increase wetting. In some of the tests leaves were selected from host plants prior to treatment and dipped in the sprays made wettable with a minimum amount of X-77. The spraying method requires less wetting agent than the dipping method and is preferred. After the spray dried, $\frac{13}{16}$-inch disks were cut from selected leaves with a cork borer and placed with the upper side down on slightly larger disks of white blotting paper. These disks were then arranged in a circle on cellulose-sponge pads in 100-mm. petri dishes partly filled with water, and then each one was infested with 10 adult mites transferred from stock plants by means of an eyebrow hair attached to a wooden handle. The petri

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[2] Roderick Sprague and Robert C. Lindner, pathologists, Washington Agricultural Experiment Station, and Hugh C. Kirkpatrick, Pathologist, Crops Research Division, Agric. Res. Serv., U.S.D.A., provided many of the chemicals; and Fay Hamilton, Field Aide, Entomology Research Division, assisted in making the tests.

[3] The chemical names of materials tested are given at the end of the article preceding "References Cited."

dishes were left uncovered, and distilled water was added daily to maintain the water barrier as an effective deterrent to migration of the mites. A few mites were lost in the water, but probably no more than would be lost by other methods that might be used to contain them. At the end of 3 days and again after 1 week, the disks were examined under the microscope to record the mortality of the adults and the number of eggs laid.

Since there was some question as to whether the mites react to treatments affecting their reproduction and development in a comparable manner on leaf disks and on growing plants, some tests were carried out on potted peach seedlings in a greenhouse. The seedlings were maintained and sprayed in the same manner as those used for leaf disks. After the spray dried the seedlings were not only supplied with mites from infested plants but were also placed near heavily infested plants. At intervals following treatment the eggs and mites were counted on 10 leaves selected at random from each plant.

Table 1.—Control of the two-spotted spider mite in the greenhouse on potted peach seedlings sprayed with cycloheximide containing pentachloronitrobenzene.

CYCLOHEXIMIDE (P.P.M.)	NUMBER PER LEAF			
	Eggs	Mites	Eggs	Mites
	Series 1			
	After 3 Weeks		*After 5 Weeks*	
200	0.2	0.2	2.4	1.2
	.8	.4	5.8	1.4
	0	0	2.3	.2
	0	0	3.8	.7
Untreated	22.3	14.6	131.2	35.2
	Series 2			
	After 2 Weeks		*After 4 Weeks*	
200	0.6	1.1	1.4	0.8
100	2.0	0.8	4.1	3.3
50	.1	1.2	4.4	2.9
25	4.3	3.0	36.5	12.1
12.5	9.7	1.9	49.2	13.2
Untreated	53.4	17.1	114.7	25.5

TREATMENTS AND RESULTS.—The effect of cycloheximide on the two-spotted spider mite on growing peach seedlings is shown in table 1. In one series of tests, egg laying was held to a low level for more than 5 weeks by the application of cycloheximide at 200 p.p.m. On leaf disks cut from these plants 5 weeks after treatment and then infested with mites, the reduction in egg laying was 96% after 3 days and 95% after 7 days. However, after the 5-week period covered by the table, the mite populations increased on the potted trees until all of them were heavily infested 3 weeks later.

In a second series of tests, different concentrations of cycloheximide were used on potted peach seedlings. Mites were prevented from increasing by all concentrations, the extent and duration of the effect increasing as the concentration increased from 12.5 to 200 p.p.m. Data presented in table 3, series 2, show that the pentachloronitrobenzene present in the cycloheximide formulation used in these tests did not significantly affect the results. The results of these two series of tests on growing peach seedlings are similar to those reported by Harries (1960) obtained by means of the leaf-disk technique described earlier. Consequently, the leaf-disk technique was used for all other tests reported herein.

Table 2.—Effect of 100 p.p.m. of cycloheximide on egg laying and mortality of the two-spotted spider mite after 4 days on different host plants.[a]

HOST PLANT	NUMBER OF EGGS PER MITE		PER CENT MORTALITY	
	Treated	Untreated	Treated	Untreated
Apple	0.2	8.7	76	24
Peach	2.6	10.9	22	6
Pear	1.3	9.3	38	26
Rose	1.2	9.2	50	28
Average	1.3	9.5	46	21

[a] Based on 5 replicates.

Cycloheximide was tested at 100 p.p.m. to afford a comparison of its effect on the numbers of eggs deposited and mortality of mites on various hosts. The tests showed that it inhibited egg laying on apple, pear, and rose, as well as on peach (table 2), reducing it by 86% on all hosts. It also killed some of the mites, but not enough to aid materially in control.

Cycloheximide was also tested to determine its ability to prevent mite increases, such as often follow the use of Sevin. In these tests, it inhibited egg laying whether it was used alone or with Sevin (table 3, series 1). Sevin did not stimulate egg laying or affect mortality of the mites.

Several derivatives of cycloheximide were also tested for activity at 50 and 100 p.p.m. at the higher concentration in comparison with several other antibiotics (table 3, series 2 and 3). According to the number of eggs laid after 3 days at 50 p.p.m., the semicarbazone was significantly less active than either the acetate or the parent compound, and pentachloronitrobenzene had no apparent effect on the activity of the parent compound. At 100 p.p.m. all the derivatives were significantly less active than the parent compound for both periods and none was outstanding. Among the other antibiotics tested, some moderate but significant inhibition of egg laying was effected by neomycin and dimethyl chlortetracycline, but less than by cycloheximide. No significant activity was shown by the other materials.

Some of the more active antiviral compounds reported by Lindner *et al.* (1959) were tested (table 3, series 4). Cytovirin was the most active material on mites, but eosin Y, 2,6,8-trichloropurine, 2,4,5,6-tetrachloropyrimidine, and 8-azaguanine caused reduction of egg laying for both periods. For the 1-week period there were also fewer eggs on leaves treated with 2-thiouracil and 6-purinethiol. Adenopterin had no effect at 20 p.p.m. in this test nor at 50 or 100 p.p.m. in later tests. These tests show that egg laying is not inhibited by all chemicals that have antiviral activity.

In another group of materials, mostly antibiotics, cytovirin again showed outstanding activity comparable to that of cycloheximide (table 3, series 5). Some phytotoxicity and toxicity to the adult mites were also shown. Egg counts indicated that Gantrisin, Achromycin, griseo-

Table 3.—Effects of some antibiotics and other chemicals on egg laying and mortality of the two-spotted spider mite on peach leaves. (10 replicates)

Treatment	Number of Eggs Per Mite After—		Per Cent Mortality After—	
	3 Days	1 Week	3 Days	1 Week
Series 1.—Cycloheximide with Sevin				
Cycloheximide, 100 p.p.m.	0.5	0.8	26	85
Sevin (50%) WP (1 lb./100 gal.):				
Alone	5.9	8.8	11	68
Plus cycloheximide, 100 p.p.m.	.6	1.0	24	81
Untreated check	6.3	10.5	7	68
Series 2.—Cycloheximide and its derivatives at 50 p.p.m.				
Cycloheximide:				
Sample A	3.0	5.1	26	76
Sample B	.4	6.6	23	79
With pentachloronitrobenzene	3.8	6.5	34	81
Cycloheximide acetate	2.1	8.4	9	70
Cycloheximide semicarbazone	6.3	20.4	13	63
Untreated check	40.3	82.2	0	36
L.S.D. at 5% level	2.7	13.4	13.7	15.3
Series 3.—Cycloheximide derivatives at 100 p.p.m. and other antibiotics				
Cycloheximide:	0.7	1.0	76	96
Acetate	4.0	6.1	19	70
Acetoacetate	4.9	9.1	25	44
Methylhydrazone	5.3	6.2	52	93
Oxime	6.8	8.4	27	77
Semicarbazone	4.4	4.9	40	90
Thiosemicarbazone	6.0	8.7	25	68
Chloromycetin (chloramphenicol), 2,500 p.p.m.	8.1	11.0	31	69
Dimethyl chlortetracycline, 1,500 p.p.m.	3.1	4.3	35	67
Neomycin, 100 p.p.m.	3.7	7.3	35	49
Phytoactin:				
100 p.p.m.	5.8	6.4	37	76
1,000 p.p.m.	6.8	9.0	43	82
Terramycin oxytetracycline, 200 p.p.m. (as hydrochloride with glucosamine)	5.2	11.0	20	42
Untreated check	6.9	12.9	9	31
L.S.D. at 5% level	2.0	3.4	19	17
Series 4.—Antibiotics and other chemicals shown to have antiviral properties (100 p.p.m. unless otherwise noted)				
Cycloheximide	1.2	1.3	21	51
Cytovirin, 50 p.p.m.	.1	.2	61	100
Eosin Y, 10 p.p.m.	1.5	3.1	30	60
2,6,8-trichloropurine	1.1	2.0	54	74
2,4,5,6-tetrachloropyrimidine	1.0	2.1	47	62
8-azaguanine	1.1	1.4	64	91
2-thiouracil	2.8	4.0	34	70
6-purinethiol	3.0	3.9	64	86
Adenopterin, 20 p.p.m.	4.9	6.9	42	71
Untreated check	4.2	6.7	12	30
L.S.D. at 5% level	1.8	2.4	9.5	16.6
Series 5.—Additional antibiotics and other chemicals				
Oleandomycin, 2,500 p.p.m.	8.5	12.6	6	80
Cytovirin, 100 p.p.m.	1.2	1.3	38	100
Gantrisin, 500 p.p.m.	6.2	9.9	15	61
Penicillin V (potassium salt), 2,500 p.p.m.	7.0	12.2	5	63
Triacetyl oleandomycin, 500 p.p.m.	5.7	10.2	15	66
Novobiocin (as acid sodium salt), 500 p.p.m.	6.6	13.2	5	34
Achromycin, 250 p.p.m.	5.1	8.7	22	64
Chlorambucil, 500 p.p.m.	5.7	9.9	30	69
Erythromycin				
1,250 p.p.m.	4.1	7.0	35	72
2,500 p.p.m.	9.8	15.7	12	77
Furaltodone, 500 p.p.m.	10.0	15.5	35	78
Griseofulvin, 2,500 p.p.m.	5.0	7.9	18	68
Nitrofurantoin, 500 p.p.m.	6.1	8.9	19	84
Polymyxin 8,300+neomycin 41,300 +gramicidin, 400 p.p.m.	5.0	8.4	33	68
Sulfadiazine, 5,000 p.p.m.	7.9	15.3	14	53
Untreated check	11.2	19.4	5	70
L.S.D. at 5% level	3.1	4.6	12.4	16.2

fulvin, erythromycin, the polymyxin-neomycin-gramidin mixture, and the anticarcinogen Chlorambucil had some activity, but too little to be promising for control. The effect of the mixture may have been due to the neomycin, which showed some activity in an earlier series. Chlorambucil is a derivative of nitrogen mustard, one of the first materials found to inhibit the multiplication of cancer cells. Sulfadiazine and nitrofurantoin, the latter used in medicine against bacterial infections, also exhibited some activity.

Both cycloheximide and cytovirin were toxic to pear seedlings at 25, 50, and 100 p.p.m. Cycloheximide did not injure apple and peach seedlings at these concentrations, but cytovirin caused brown spotting of some of the leaves. Probably many plants will not tolerate either of these materials in sufficient strength for mite control. Their practicability for the control of mites on fruit trees must be shown by field tests on different varieties and under different weather conditions. Whatever the outcome of field tests, it is important to know that mites can be controlled by inhibition of egg laying.

Chemical Names of Materials Tested

Achromycin—tetracycline hydrochloride
Adenopterin—*N*-[*p*-([1-(2,4-diamino-6-pteridyl)ethyl] methylamino) benzoyl] glutamic acid
Chlorambucil—4-(*P*-(bis-2-chlorethyl) (amino) phenyl) butyric acid
Cycloheximide—3-[2-(3,5-dimethyl-2-oxycyclohexyl)-2-hydroxyethyl] glutarimide
Erythromycin—propionyl ester of lauryl sulfate
Furaltodone—5-morphalinomethyl-3-(5-nitrofurfurylideneamino)-2-oxyazolidinone
Gantrisin—N′-3,4-dimethyl-5-isoxazolyl.
Nitrofurantoin—1-(5-nitro-2-furfurylidene amino) hydantoin
Sevin—1-naphthyl methylcarbamate.

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Incidence of Field Spread of Internal Cork of Sweet Potato in Insecticide-Treated Plots[1]

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ABSTRACT

Field plots of originally internal cork-free sweet potato plants were treated with insecticides in 1955–59 in Louisiana to reduce spread of internal cork by controlling the principal vector, *Aphis gossypii* Glover. Amounts of disease spread during the season from adjacent cork-affected sources were proportional to vector activity. Reduction of the disease ranged from 0% to 50%.

Isolation of virus-free plantings of sweet potato seed stocks is the means presently employed for combatting field spread of internal cork virosis in commercial plantings (Martin & Kantack 1958a, 1960).

Success in reducing field spread of a virus disease with insecticides is greatest when the vector is controlled before it leaves the source plant (Smith & Brierley 1956). However, the best means to employ will be determined by mode of transmission of the virus by the vector. Broadbent (1957) discusses usefulness of insecticides in control of virus spread.

Spread of internal cork from sweet potato to sweet potato gave evidence of transmission as a nonpersistent virus (Martin & Kantack 1958b). Winged forms of the cotton aphid, *Aphis gossypii* Glover, dispersing into sweet potato fields were shown to be the principal vector in Louisiana (Kantack *et al.* 1960). Sweet potato is a poor host for these aphids. Being restive on sweet potato they move about freely, thus spreading the virus from plant to plant.

Insecticide treatments of sweet potatoes in field plantings were initially begun in Louisiana in 1955 to study possible internal cork virus-vector relationships. At that time the method of spread of internal cork was not known. Spread of internal cork into cork-free plots was reduced 50% by foliar applications of DDT and American Cyanamid Compound 12008 (*O-O*-diethyl *S*-isopropylthiomethyl phosphorodithioate) made in an experiment where plots of cork-affected plants were included in a randomized block (Kantack & Martin 1956). These data are summarized in table 1 (Experiment 1). Where cork-free plantings were isolated 100 yards or more from cork-affected plantings, no reduction in spread of the disease was obtained by dusting with a mixture of DDT and malathion or by spraying with phorate (Martin & Kantack 1958a, 1960). However, the amount of internal cork infection introduced in these experiments was minimal. It was recognized as early as 1956 that inoculation from plant to plant was occurring at relatively short distances (Martin & Kantack 1958b). Subsequent tests were designed in latin squares to obtain uniform plot infection as discussed by Broadbent (1957). Results given herein are on spread of internal cork in the field in experiments made in 1955, 1956, 1957, 1958 and 1959 to control the vector with insecticides.

METHODS OF PROCEDURE.—Plots were three rows wide and 40 to 45 ft. long with 10-ft. buffer zones between blocks. Plants of the Unit 1 Porto Rico variety were set 14 in. apart in rows 4 ft. wide about June 1. Source of cork-free plants was from an isolated stock which never had shown internal cork symptoms in the roots. This stock did, however, have a history of chlorotic spotting and purple-ring spotting in the leaves. Source of

Table 1.—Mean[a] percentages of internal cork-affected roots and mean disease indices in tests to determine suppression of the vector with insecticides.

LOCATIONS, PLANTING STOCKS, AND TREATMENTS	CORK-AFFECTED ROOTS			
	Percentage		Disease Index	
	At harvest	After storage	At harvest	After storage
Experiment 1. L.S.U. 1955				
Cork-affected	42.3	91.0	25.9	77.0
Cork-free	5.2	54.8	3.2	38.3
Cork-free+DDT dust+AC-12008 spray on foliage	2.7	31.3	1.3	18.3
L.S.D. at 5% level	5.94	15.74	4.28	14.05
at 1% level	8.47	22.37	6.09	19.97
Experiments 2 and 3, 1956[b]				
Cork-affected	41.4	60.9	29.1	41.3
Cork-free	14.2	14.8	9.5	8.3
Cork-free+AC-12008 spray in soil	15.2	19.6	10.1	11.7
Cork-free+demeton spray on foliage	9.4	16.3	5.8	9.6
Cork-free+DDT dust on foliage	9.0	11.8	4.4	6.8
L.S.D. at 5% level	8.99	10.57	6.80	6.31
at 1% level	12.61	14.84	9.55	8.85
Experiment 4, 1956				
Cork-free	8.5	7.8	5.0	3.2
Cork-free+DD mixture in soil	6.2	6.0	3.6	3.2
Cork-free+MC-2 in soil	13.4	13.8	8.0	7.3
Cork-free+dieldrin spray in soil	5.8	7.5	3.6	4.1
L.S.D. at 5% level	5.37	n.s.	2.93	n.s.
at 1% level	n.s.	n.s.	n.s.	n.s.
Experiment 5, 1957				
Cork-affected	24.5	50.0	14.5	36.4
Cork-free	0.8	2.7	0.4	1.6
Cork-free+DDT dust on foliage	0.7	2.6	0.4	1.9
Cork-free+demeton spray in soil	0.3	3.2	0.1	1.4
L.S.D. at 5% level	6.26	7.86	3.14	10.10
at 1% level	8.99	11.30	4.52	14.53
Experiments 6 and 7, L.S.U. 1958–59[c]				
Location A				
Cork-affected	27.8	89.1	16.7	59.0
Cork-free	0.3	2.5	0.1	1.1
Cork-free+phorate spray on foliage	1.3	3.6	0.8	1.7
Cork-free+DDT dust on foliage	1.5	3.2	0.9	2.1
L.S.D. at 5% level	5.64	4.73	4.55	3.68
at 1% level	8.11	6.80	6.54	5.29
Location B				
Cork-affected+phorate spray on foliage	37.6	82.5	21.2	56.5
Cork-free	1.6	6.0	0.5	2.5
Cork-free+phorate spray on foliage	0.7	4.5	0.2	1.8
Cork-free+DDT dust on foliage	1.6	9.0	0.4	4.8
L.S.D. at 5% level	5.35	15.40	3.31	15.33
at 1% level	7.69	22.15	4.75	22.04

[a] Mean of 6 replications in Experiment 1, and of 4 replications in each of the others.

[b] Combined data from a test at Baton Rouge, Louisiana, and a duplicate test at Opelousas, Louisiana.

[c] Data combined for years 1958–1959 in split location tests (Locations A and B) separated 300 feet or more. The r values for comparison of per cent of internal cork-affected roots at locations A and B were 0.9810 at harvest and 0.9633 after roots were stored. Respective r values for severity of root lesions were 0.9186 and 0.9430.

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cork-affected plants was from roots showing internal cork lesions.

In 1955 cork-free sweet potato plantings were dusted twice weekly with 10% DDT at a rate of 10 pounds per acre during the entire growing season. Am. Cyanamid 12008 also was applied to the same plots at a rate of 2 pounds per acre once weekly in a water-spray formulation from mid-June to mid-July. In addition to DDT foliage-dusted plots as in 1955, the 1956 experiments were expanded to include a treatment with demeton in a water-spray formulation applied to the foliage once weekly at a rate of 1 pound per acre. A preplanting soil treatment plot made with Am. Cyanamid 12008 at 8 pounds per acre was included in this experiment. An additional experiment included DD® (1,3-dichloropropene and 1,2-dichloropropane mixture) at 15 gals./acre, MC-2 (methyl bromide) soil treatment at 1.5 lb./100 sq. ft. of surface area, and dieldrin mixed in the soil at an 8 lb./acre rate. Demeton was applied to the soil at a rate of 8 lb./acre in a preplanting treatment in 1957 for comparison with cork-affected plantings and cork-free, DDT-treated plots included in experiments each year.

In 1958 and 1959 tests were in locations separated by at least 300 feet. Cork-affected and cork-free plantings were made at each location. Every other week foliage of cork-free plants was sprayed with phorate at a rate of 1 lb./acre. Cork-affected plantings were treated in like manner at one location. This design was made to study suppression of the cotton aphid, the principal vector, before arrival at the suscept. Additional information sought was the influence of vector activity on cork lesion development in roots from originally cork-affected plantings.

Intermingling of foliage between treatments in the experiment was prevented by throwing the vines back into their respective plots at frequent intervals. In most cases root samples were obtained from the center row of each plot. At harvest and after storage for 5 months at about 75° F., 1 bushel of roots from each plot was examined for internal cork lesions. Each root was cut into $\frac{1}{16}$-in. slices and classed into one of 5 classes: from 0 indicating no cork lesions to 4 indicating severe cork lesions. Mean disease indices were calculated by the following formula:

$$\frac{\text{Sum of (number of roots in each class} \times \text{class number)}}{4 \times \text{number of roots examined}} \times 100$$

RESULTS AND DISCUSSION.—Greater aphid activity occurred during 1955 and 1956 than in succeeding seasons. No record was made of aphid flights during the 1955 season. However, since the cotton aphid was abundant on adjacent cotton plantings in 1955, it could be expected that sufficient numbers of these aphids in flight would coincide with the lush growth period of sweet potato plants grown in the test that year (Experiment 1, table 1).

Greatest dispersion of *A. gossypii* in 1956 was during late June and early July. Significant increase of internal cork in roots at harvest from soils treated with MC-2 as compared with other treatments (Experiment 4, table 1) indicates greater vector activity resulted on plants in MC-2-treated soils, or the treatment rendered plants more susceptible to infection. Plants in this treatment had an abnormally yellow color in the leaves during the lush growth period. Because of the yellow color of the foliage, aphids were probably attracted to them in greater numbers than to plants in the other treatments. Plants seem thus to have acquired a concentration of virus sufficient to induce root lesions more readily. Extended latent periods for root lesion development after inoculation have been apparent in tests when vectors are less active (Kantack *et al.* 1960). More time seems to be required for virus concentration to develop to sufficiently high levels to produce symptoms after a minimal inoculation of virus.

Roots from the 1956 experiments examined after storage showed little increase in number with internal cork lesions when compared with those examined at harvest. However, roots from the 1956 tests were stored at temperatures below 75° F. Storage conditions for this season were thus less favorable for increase of internal cork lesions than in the tests in other years. It is established that temperature greatly influences root lesion development (Aycock *et al.* 1953).

Plants in the experiments in seasons subsequent to 1956 were not subjected to intense activity of the vector as in the previous seasons. Consequent low amounts of internal cork spread during these years are more difficult to evaluate. Less aphids were in flight during the 1957 season (Experiment 5) than for other seasons, and a correspondingly smaller amount of infection resulted. Also, small amounts of internal cork spread into cork-free check plots during 1958 and 1959 (Experiments 6 and 7), but vector activity was some greater for these seasons than in 1957. There was a high correlation for amount of internal cork at locations (A and B), and a high correlation for severity of lesion development. Differences between percentage of roots with internal cork or severity of lesions in originally cork-affected plantings in split locations were not significant.

Mean percentage reduction of cork-affected roots resulting from insecticide treatments in originally cork-free plots ranged from 0% to near 50%. Reduction apparently was influenced by magnitude of vector activity as well as by the insecticides and methods of application.

Casual winged insect vectors which may alight and feed on a plant before moving off again are more important agents in the spread of a virus than vectors which alight and remain to colonize the crop (Kennedy 1950). When such agents must necessarily be suppressed in a short interval of time to prevent spread of a nonpersistent virus from plant to plant, as appears to be the case with internal cork of sweet potato, control of disease spread by controlling the vector is difficult.

Spraying with insecticides had little measurable effect on spread of internal cork in field plantings when small amounts of the disease were introduced from a distance into cork-free plantings (Martin & Kantack 1958a, 1960). Insecticides gave near 50% control of field spread in cork-free plantings when more than one-half of the plants in comparable checks became infected from source plantings made in the same tests. Little or no control was evident when magnitude of spread into cork-free check plots was at a minimum.

Success in controlling internal cork of sweet potato with insecticides probably will depend upon the use of quick-killing, long-lasting toxicants, or other materials which will prevent the aphids from probing or feeding, since the majority of spread is from plant to plant within the crop.

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Hazards Associated with the Implantation of Tetram into Elm Trees for Dutch Elm Disease Control[1]

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ABSTRACT

Tetram® (*O,O*-diethyl *S*-(β-diethylamino)-ethyl phosphorothiolate hydrogen oxalate) administered by trunk implantation is effective in preventing infection of elm trees with the Dutch elm fungus through controlling the bark beetle vector. Tetram is of such high general toxicity to animals and is so persistent in the elm that several hazards would be associated with its use for Dutch elm disease control. Leaves dropping in the fall from trees treated in the spring are very toxic to mammals and earthworms ingesting them, and the smoke generated on burning such leaves can effect cholinesterase depression in mammals.

It has been demonstrated that implantation of the experimental insecticide Tetram® (*O,O*-diethyl *S*-(β-diethylamino)-ethyl phosphorothiolate hydrogen oxalate) into the trunk of an elm tree can effect control of *Scolytus multistriatus* (Marsh.) (Al-Azawi & Casida 1958). Tetram is localized in elm bark tissue in amounts sufficient to reduce the extent of feeding on twig bark so that this beetle vector of the Dutch elm disease fungus, *Ceratocystis ulmi* (Buis.) Moreau, dies or stops feeding before reaching the infection site (Al-Azawi & Norris 1959). Since Tetram is known to be highly toxic to mammals (Plant Protection Ltd. 1957), the hazard associated with its potential use as a systemic insecticide for combatting Dutch elm disease was investigated.

METHODS AND RESULTS.—*Leaves from Tetram-Treated Trees*—Forest-inhabiting, 2-inch d.b.h. elms (*Ulmus americana* L.) were implanted with 10 grams technical Tetram per tree by placing a 50% aqueous solution of the insecticide in three holes drilled in the trunk according to a described procedure (Al-Azawi & Norris 1959). Leaves from this set of 10 trees treated on May 8, 1959, were used throughout the experiments described. At three different times during the season, leaves were collected and analyzed for anticholinesterase agents (Casida *et al.* 1956) and the results were interpolated from standard curves prepared with Tetram to obtain the p.p.m. Tetram in the foliage. The p.p.m. found were 1,796 at 26 days, 5,058 at 143 days and 3,796 at 164 days after treatment based on the wet weight of the leaves. This last value was the Tetram content of the leaves when they dropped in the fall. At the time of leaf drop, a portion of the leaves was immediately frozen and held at −5° C. The remainder was allowed to air-dry outside for 76 days (*i.e.*, 240 days total after treatment of the trees), and then these dried leaves, which analyses showed to contain 8,431 p.p.m. Tetram based on the dry-leaf weight, were held at −5° C. until used.

Leaching of Tetram from Leaves and Stability of Leached Material.—One gram of whole leaves from treated trees at the time of leaf drop was placed in 100 ml. distilled water in a flask. These leaves contained 3,796 p.p.m. Tetram. Three such flasks were held at 30° C. and three other flasks were held at the transient outside temperature from the first week of December until the first week of April. At different intervals small aliquots of the water were analyzed for Tetram leached from the leaves. Within 1 day sufficient Tetram had leached from the leaves to yield 24 to 26 p.p.m. Tetram in the water and the pH of the water was about 5.35. The material held outside dropped to about 12 to 15 p.p.m. in the water by 30 days, at which time the water froze. No further change in Tetram concentration occurred during the 80 days it remained frozen outside, and the pH did not change from the 6.65 value recorded when the water froze. The

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Table 1.—Effect on rats of diets containing leaves from Tetram-treated elm trees.

Diet (biscuits)	Days on Diet	Av. Daily Food Intake (gm.)		Av. Daily Weight Change as Per Cent of Initial Weight	Organophosphate Poisoning Symptoms	ChE Inhibition at End of Experiment (%)		
		Total	Tetram Leaves			Brain	Plasma	Corpuscles
Alone	14	18	0	+5.1	None	—	—	—
Containing 20% control leaves	14	17	0	+4.5	None	—	—	—
Containing 1.25% Tetram leaves	14	10	.125	0	Mild	62	95	86
Containing 5% Tetram leaves	7	2.8	.14	−7.4	Severe[a]	46	95	77
Containing 20% Tetram leaves	6	.7	.14	−9	Severe[b]	—	—	—

[a] 1 died on seventh day.
[b] 1 died fifth day and 2 died on sixth day.

leaves held at 30° C. reacted in the same way as those held outside for the first 30 days, but then molds and other microflora caused the pH to rise with a subsequent increase in the rate of Tetram destruction. The Tetram content of the water fell from 8 p.p.m. at 34 days to 3 p.p.m. at 55 days, 0.6 p.p.m. at 69 days, 0.05 p.p.m. at 89 days and 0.005 p.p.m. at 110 days when the pH was about 8.5. It appears that Tetram can be partially leached from leaves and that the leached material is very stable as long as the leachate is acid and/or low in content of molds and other microflora.

Rats Fed on Leaves From Treated Trees.—Diets were prepared for rats by mixing various proportions of ground dog biscuit (Garver's Supply Company, Inc., Madison, Wisconsin) with a leaf powder prepared from the dried leaves containing 8,431 p.p.m. Tetram. The diets contained 1.25%, 5% or 20% of their weight as leaves based on the leaf weight before drying. A 20-gram lot of each mixture was made into a thick paste by adding water, and this mixture was given to each of three male albino rats each weighing 160 grams. Control rats were fed diets either of 20 grams of leaves from untreated trees or on the dog-biscuit paste alone. Since the control rats consumed their total ration each day, they were given new food daily while the animals receiving leaves from treated trees in their diet received new ration every 1 to 3 days. Any food left was dried and weighed to determine the amount consumed. In addition, rats were weighed daily and symptoms of organophosphate poisoning noted.

On sacrifice, tissues from the rats were assayed for cholinesterase (ChE) activity by a described manometric procedure (Seume *et al.* 1960). After centrifuging the blood to remove the plasma, the corpuscles were resuspended in buffer to the original blood volume. The tissue levels used per flask with a 2.0-ml. total-reaction mixture were 30 mg. brain, 200 μl. plasma and 400 μl. reconstituted corpuscles. Substrates at 1×10^{-2} *M* were propionyl choline for the plasma and acetyl-β-methylcholine for the corpuscles and brain. The control b_{30} values (μl. CO_2 evolved per 30 minutes at 38° C.) were 80 for the brain, 114 for the plasma and 69 for the corpuscles.

Rats fed on dog biscuits containing 20% elm leaves from untreated trees were comparable to rats with no elm leaves in their diet in respect to food intake and weight gain. As little as 1.25% leaves from treated trees in the diet produced mild symptoms of organophosphate poisoning and marked cholinesterase depression. The higher levels of treated leaves in the diet produced severe symptoms or death in a few days, even though only small amounts of these diets were consumed by the rats (table 1).

Mice Exposed to Smoke From Burning Leaves of Treated Trees.—Leaves from treated trees were burned in an apparatus so designed that smoke generated in a burning chamber was diverted into a mouse chamber and then into two successive smoke-trapping containers. The tandem of four 500 to 1,000 ml. Erlenmeyer flasks was connected by glass tubes fitted with ground-glass joints. The first flask was a burning chamber with a 50-mm. opening near its bottom for insertion of a Nichrome coil connected to a variable condenser for heating the wire. The coil was placed into the flask and the opening packed loosely with glass wool. The second flask was a mouse chamber, and the other two contained 150 ml. each of 1,000 p.p.m. oxalic acid solution to trap the smoke passing out of the mouse chamber to bubble through the solutions. The rate of air flow through the tandem was controlled with a water pump attached to the outlet of the last flask. By carefully adjusting the temperature of the Nichrome coil, dried leaves from control trees could be almost completely burned yielding a dense smoke which greatly irritated the mice but did not appear to otherwise affect them adversely.

Three grams of leaves, either from control or treated trees or mixtures of leaves therefrom, were burned and two 25-gram female albino mice were exposed to the smoke. In order to prevent suffocation of the mice by the smoke, the leaf samples were burned in three 1-gram portions so that the total time for burning was 10.5 minutes. The mice were then held for 2.5 hours (average from 2 hours in one run and 3 hours for another run) before sacrifice.

The brain, corpuscles and plasma of the mice were assayed for cholinesterase activity as described above. The tissue levels were 80 mg. brain, 25 μl. plasma and 200 μl. reconstituted corpuscles per flask. The control b_{30} values at 38° C. were 88 for plasma, 28 for corpuscles and 125 for brain.

In addition, the Tetram content of each of the flasks in the burning and smoke-scrubbing tandem was determined. Since the ash from burning the leaves yielded a pH of 10 to 11 on adding it to water, it was immediately neutralized with hydrochloric acid to prevent further

Table 2.—Degradation of Tetram on burning elm leaves from treated trees and effect of the smoke on mice.

TETRAM LEVEL OR EFFECT ON MOUSE	GRAMS OF TREATED LEAVES BURNED (PLUS CONTROL LEAVES TO 3 GM.)		
	1	2	3
Total μg. Tetram before burning	8,431	16,862	25,293
Total μg. Tetram after burning			
Flask 1.—Ash[a]	0.6	1.8	1.7
Flask 2.—Rinse from mouse container[b]	.4	.7	.8
Flask 3.—Bubbled through water[c]	.5	1.2	1
Flask 4.—Bubbled through water[c]	.5	.9	1
Mouse plasma ChE inhibition (%)[d]	15	36	53

[a] Weight of ash averaged 0.82 gm. from burning 3 gm. dried leaves.
[b] After mice were removed, the flask was rinsed with 50 ml. water to recover Tetram for assay.
[c] Each flask with 150 ml. of water containing 1,000 p.p.m. oxalic acid.
[d] No cholinesterase inhibition was detected in the brain or corpuscles.

Tetram breakdown before analysis.

Almost all of the Tetram was destroyed during this burning process (table 2). Although only a few micrograms of Tetram were left in the ash or trapped in the scrubbers, sufficient Tetram was inhaled by the mice to effect plasma cholinesterase inhibition.

Earthworms Exposed to Leaves From Treated Trees.—The toxicity of leaves from Tetram-treated trees to earthworms was studied both in aqueous solution and in soil. Petri-dish tests were made by placing a filter paper, 5 ml. water or Tetram-solution and 10 earthworms (*Lumbricus terrestris* L.) into a 10-cm. dish. The earthworms averaged 0.5 gm. each in weight. Concentrations were varied by a factor of two- to three-fold, 10 replicates were used at each concentration and the mortality was recorded after 24 hours. After correction of the mortality data by Abbott's formula (Abbott 1925), the LD_{50} was estimated from logarithm-probit mortality plots. An LD_{50} value of 6 to 7 p.p.m. was found for aqueous solutions of Tetram. Varying amounts of the finely ground leaf powder containing 8,431 p.p.m. Tetram were also assayed in petri-dish tests. If the concentration of Tetram in the assay was calculated on the basis of all the Tetram from the leaf powder going into solution in the 5 ml. water, then the LD_{50} value was again 6 to 7 p.p.m. Tetram. Both the LD_{50} values and the slopes of the mortality curves were similar for the Tetram solutions and the suspended powder from Tetram-containing leaves. This indicates that with finely powdered leaves the Tetram was largely leached from the leaves to yield a leachate as toxic to earthworms as a comparable amount of Tetram added directly to water.

A closer approximation to natural conditions was also used in evaluating the toxicity of leaves from Tetram-treated trees to earthworms. Each of 6 wooden flats (14″×20″) was divided into 3 equal sections by 2 wooden partitions. Each section was filled to a depth of 3 inches with compost soil (3 kilogram dry weight). The soil was moistened with water, 24 hours later 25 earthworms were added to each section, and 5 days later 3 flats were treated with 6 grams of Tetram-containing leaves (5,058 p.p.m. Tetram in the leaves) per section while the other 3 flats received the same amounts of leaves from control trees. The leaves used were comparable to those dropping from trees in the fall, and the whole leaves were distributed uniformly over the soil surface. Each morning 100 ml. water was sprinkled over the leaves in each section. At 5, 10 and 15 days, soil was removed from one section of each flat and the mortality recorded. The mortality, after correction by Abbott's formula, was 42% at 5 days, 60% at 10 days and 90% at 15 days.

CONCLUSION.—Tetram is so stable within elm trees and so toxic to a variety of organisms that it cannot be recommended for general use in Dutch elm disease control despite the unique activity of this chemical as a systemic for control of *Scolytus multistriatus*.

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Susceptibility of an Eye Gnat, *Hippelates pusio*, to Residual Insecticides[1]

ANDREW SPIELMAN[2]

ABSTRACT

A convenient method of measuring the susceptibility of adults of *Hippelates pusio* Loew to residual insecticides was developed. With this technique hourly 50% knockdown and kill concentrations for DDT, malathion, dieldrin and lindane were estimated for a Cuban population of *H. pusio*.

Lindane was the most promising of the insecticides tested because of its rapid action in low concentrations. Dieldrin and malathion were slower. DDT was ineffective for the population tested and may be generally ineffective against *Hippelates*.

Eye gnats of the genus *Hippelates* may be annoying pests as well as vectors of disease, and have proved difficult to control. While soil larviciding as a control measure has been studied extensively and actually employed with some success (Mulla 1959), adulticides have not received much attention.

A lindane thermal aerosol had little effect upon caged *H. collusor* adults (Rees 1952) but malathion appeared to produce some kill of *H. pusio* (Mulla 1959). In an unpublished study, Tinkham (1948–1949) found that lindane was effective as a space-spray upon caged *H. pusio* adults and that dieldrin and chlordane were less so. His data show that DDT was a very poor adulticide. Field trials of these and other insecticides in thermal aerosol applications did not produce measurable effects. Another unpublished report (Turner 1960) describes a wind-tunnel device for evaluating space-sprays and two methods of testing the residual effects of insecticides. Again, DDT appeared to have little effect upon *H. pusio*.

In the present study, a method for the testing of the susceptibility of *Hippelates* flies to residual insecticides was developed, and the effects of four common insecticides upon Cuban *H. pusio* Loew were observed.

MATERIALS AND METHODS.—In 1959, *H. pusio* females were netted from horses stabled at Guantanamo Bay, Cuba. Large quantities of insecticide, especially DDT, had been routinely used in the area by the U. S. Navy for more than 10 years. The insecticide was not applied for eye gnat control, however, and it is possible that these insects were not exposed to significant amounts. In addition, females of *H. impressus* Becker were collected from man in an area near Guantanamo Bay where insecticides had never been used.

The gnats were immediately transferred to screw cap vials (12 mm. × 100 mm.) in which a water-saturated charcoal-plaster mixture was slanted. Each vial contained 10 gnats and was stored in the insectary at 25° ± 2° C. for 2 hours. At that time, any dead gnats were removed and the insecticide exposure begun. The gnats could be handled without anesthesia.

Just prior to use, insecticide-treated papers were prepared from quarter-inch strips of Whatman #5 filter paper. The strips were 3½ inches long and were marked at 1-inch intervals from one end. Acetone solutions of standard insecticides were freshly prepared in serial ten-fold dilutions in concentrations from 10.0% to 0.0001% and the papers dipped into the appropriate solution to the first inch mark. The strips were left in the solution until the liquid rose to the second mark and were then hung in a vertical position at insectary temperature until dry. A half-inch tab was made at the untreated end of the paper by folding at the third inch mark.

The gnats were exposed by placing a dried paper in each vial with the cap screwed down on the folded tab. The vials were then set aside in a quiet corner of the insectary for 15 minutes, at which time the papers were removed and the first reading of knockdown and kill were made. Subsequent readings were made at 1, 2, 3, 6 and 24 hours after the beginning of exposure. Knockdown was considered to have begun when an insect lost equilibrium and was no longer able to crawl over the sides of the vial in a sustained manner. Kill was judged by the failure of an insect to move when the vial was agitated. The insects remained active while being exposed to the treated papers, moving freely over the papers and the sides of the vials. Each test included two or more concentrations of each of the four insecticides and a control vial exposed to an acetone-treated control paper. Approximately 200 females of *H. pusio* were exposed to each of the insecticides.

RESULTS.—While there was virtually no mortality during the 2-hour pre-exposure holding period, beginning at 3 hours after insertion of the acetone-treated control papers into the vials, some knockdown and kill occurred (table 1). Death followed soon after knockdown in the control vials.

The insecticide concentrations that produced 50% knockdown and kill were estimated graphically (table 2).

Table 1.—Knockdown and kill of adults of *H. pusio* exposed to acetone-treated papers without added insecticides.[a]

Criterion of Activity	Per Cent Showing Effect					
	Hours After Exposure					
	0.25	1	2	3	6	24
Knockdown	0	0	0	5.0	31.3	57.8
Kill	0	0	0	1.3	13.8	43.8

[a] Based on 80 females.

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Table 2.—Calculated concentrations of DDT, malathion, dieldrin and lindane that produced 50% knockdown and 50% kill with Cuban *H. pusio*.

	Calculated Concentration in Per Cent					
	Hours After Exposure					
Insecticide	0.25	1	2	3	6	24
			50% Knockdown			
DDT	>10.0	>10.0	>10.0	>10.0	8.0	0.3
Malathion	>10.0	0.4	0.1	< 0.01	< 0.01	<0.01
Dieldrin	>10.0	0.004	0.001	< 0.0001	< 0.0001	<0.0001
Lindane	0.07	0.0007	0.0001	< 0.0001	< 0.0001	<0.0001
			50% Kill			
DDT	>10.0	>10.0	>10.0	>10.0	>10.0	0.4
Malathion	>10.0	>10.0	0.8	0.2	0.08	<0.01
Dieldrin	>10.0	>10.0	>10.0	0.001	< 0.0001	<0.0001
Lindane	> 2.0	0.2	0.009	0.0004	< 0.0001	<0.0001

The control readings were adjusted with Abbott's formula (Busvine 1957). DDT failed to produce a significant knockdown within 6 hours of exposure to the strongest concentrations used and the interval between knockdown and death was as long as 12 hours. Lindane, on the other hand, was rapidly effective, even in low concentrations, with a marked immediate knockdown. The action of dieldrin was delayed, with low concentrations producing a high rate of knockdown within 1 hour. Malathion had a slower action than dieldrin, but was far more rapid than DDT. Fairly high concentrations of malathion were required to produce a 50% knockdown at 1 hour. The interval between knockdown and kill was 2 to 3 hours for the three latter insecticides.

An attempt to utilize *H. impressus* in a series of similar tests was not successful because of the poor survival of control insects. The pattern of knockdown with the four insecticides was similar to that observed in the experiments with *H. pusio*.

Discussion.—Lindane appears to be an effective laboratory adulticide for *H. pusio* and DDT a very poor one. In the confined space within the tube, however, the strong fumigant action of the former undoubtedly reinforces its contact effect, perhaps thus invalidating the comparison of these chemicals for outdoor use. The effect of minute amounts of lindane is so striking, however, that it cannot entirely be attributed to vapor toxicity. Tinkham's space-spray results support this contention.

In setting up a barrier strip or in treating the premises in which a pest problem exists, it is essential that the adulticide have a rapid knockdown action from which the insect does not recover. Lindane acts in this way under the conditions described. Dieldrin and malathion take effect more slowly and DDT so slowly that it would appear to be ineffective against the population of *H. pusio* studied.

It is not known to what extent the general use of DDT in the study area may have affected the susceptibility of *H. pusio*. *H. impressus*, collected from an area in which no insecticides had been used, however, demonstrated a similar pattern of relative susceptibility to the four insecticides, and both Tinkham's and Turner's data indicated a lack of susceptibility to DDT.

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The Development and Characterization of Resistance to Carbamate Insecticides in the House Fly, *Musca domestica*[1]

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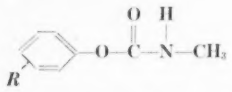
Abstract

Two subcolonies of a house fly strain of near normal susceptibility to all insecticides except to members of the cyclodiene group were selected with Isolan® (1-isopropyl-3-methylpyrazolyl-(5)-dimethylcarbamate) and Compound III (3-isopropylphenyl *N*-methylcarbamate), respectively, during all life stages. After 20 generations of selection with Isolan, a 7- and 19.5-fold resistance to this compound was present in adults and larvae, respectively. Selection with Compound III resulted, respectively, in >50- and 7.5-fold resistance in these stages. Injection of Compound III indicated a 4.9-fold level of adult resistance.

Cross-resistance in the selected strains was nearly complete to carbamates of the general formula



where R- was either 2-isopropyl, 3-isopropyl, 2-isopropoxy, or 3-*tert*-butyl; to Pyrolan® (1-phenyl-3-methylpyrazolyl-(5)-dimethylcarbamate); and to Sevin® (1-naphthyl *N*-methylcarbamate). Cross-resistance to 3,5-dimethoxyphenyl *N*-methylcarbamate and phenyl *N*,*N*-dimethylcarbamate was approximately 10- and 3-fold, respectively. Both carbamate-selected strains showed low cross-tolerance (<3-fold) to DDT, Prolan® (1,1-bis (*p*-chlorophenyl)-2-nitropropane), parathion, Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O*,*O*-dimethyl phosphorothioate) and dicapthon, and higher cross-tolerance or low cross-resistance (3-to 6-fold) to methoxychlor, malathion, Diazinon® (*O*,*O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), ronnel and allethrin.

Chlorinated hydrocarbon and organophosphorus insecticide-resistant strains manifested cross-resistance levels toward carbamates approximating those exhibited by the carbamate-selected strains. In all cross-resistance tests, some specificity within the *N*-methyl and *N*,*N*-dimethyl carbamate groups was evident.

Cross-tolerance to either Isolan or Compound III in combination with piperonyl butoxide (1:5 ratio) was <6- and <3-fold, respectively, in all resistant strains, regardless of history of exposure. Selection of the Compound III-resistant strain for eight additional generations with Compound III plus piperonyl butoxide resulted in only threefold tolerance to this combination.

Among the new types of insecticides, the carbamic acid esters (Gysin 1952, 1954, Kolbezen *et al.* 1954, Metcalf *et al.* 1960) have attracted much attention, especially since the introduction of Sevin® (1-naphthyl *N*-methylcarbamate) (Haynes *et al.* 1957) as an agricultural insecticide. Since several carbamates are efficient house fly toxicants, the question of house fly resistance to these compounds naturally arises.

Information on resistance to carbamates is limited and in certain respects contradictory. Lack of sufficient work on this problem may be attributed to a tendency to assume similarity of resistance development toward carbamate and organophosphorus insecticides, because of the relation of these two groups as cholinesterase inhibitors. The present study, however, indicates some important differences in the character of resistance developed toward these two groups of insecticides.

Meltzer (1956) selected a susceptible strain of house flies with phenyl *N*,*N*-dimethylcarbamate for 28 generations and reported only a slight increase in tolerance to this material. After releasing the selection pressure, this tolerance was not lost within the next 23 generations (Meltzer 1960). Wiesmann (1956) subjected a susceptible strain to Pyrolan® (1-phenyl-3-methylpyrazolyl-(5)-dimethylcarbamate) pressure for 2 years and reported a 300-fold resistance to this compound. Since the present study was begun, the selection experiments of Moorefield (1960) with carbamate insecticides were disclosed. This author subjected four subcolonies of the Chemical Specialties Manufacturers Association susceptible strain to 70% adult pressure with 3-isopropylphenyl *N*-methylcarbamate, 3-*tert*-butylphenyl *N*-methylcarbamate, Sevin and Pyrolan, respectively. Selection with Sevin and 3-*tert*-butylphenyl *N*-methylcarbamate produced strains virtually immune to these compounds. However, selection with Pyrolan for 26 generations and with 3-isopropylphenyl *N*-methylcarbamate for 67 generations resulted in only 7-fold and 8-fold levels of resistance, respectively.

Materials and Methods.—*Terminology.*—The term "resistance" as employed in this paper denotes an increase in LD_{50} value of five or more times that of the normal. The term "tolerance" has been applied to increases of lower magnitude.

Insecticides.—The materials employed were highly purified by recrystallization or distillation. Isolan® (1-isopropyl-3-methylpyrazolyl-(5)-dimethylcarbamate) (b. p. 86° to 90° C. at 0.15 mm.) and Pyrolan (m. p. 50° C.) were supplied by J. R. Geigy, Basel, Switzerland; Sevin (m.p. 142° C.) by Union Carbide Chemicals Company, New York; Compound I (S.17) (phenyl *N*,*N*-dimethylcarbamate) (m.p. 44° to 46° C.) by Philips-Duphar, Amsterdam, Holland; and Compound III (AC-5727) (3-isopropylphenyl *N*-methylcarbamate) (m.p. 72° to 73° C.) by Hercules Powder Company, Inc., Wilmington, Delware. The other substituted phenyl *N*-methylcarbamates, described in previous reports from this laboratory (Kolbezen *et al.* 1954, Metcalf *et al.* 1960), will be referred to in this article by the following Compound numbers: Compound II (2-isopropyl-), Compound IV (2-isopropoxy-), Compound V (3,5-dimethoxy-), and Compound VI (3-*tert*-butyl-). More complete definitions of Compounds II, IV, V and VI are given in table 6.

House Fly Strains Employed.—The laboratory strain ($S_{-Lab.}$) which has been maintained at the Citrus Experiment Station, Riverside, for a number of years (March & Metcalf 1949) without exposure to insecticides, has

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been used as parental in the present selections. This strain undoubtedly possesses a rich reservoir of genetic potential for development of resistance, as evidenced by the fact that strains resistant to DDT, methoxychlor, Prolan® (1,1-bis(*p*-chlorophenyl)-2-nitropropane), lindane, dieldrin, parathion, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate) and malathion have already been produced from it (March 1959). At the beginning of the present study it was found that this strain had developed extremely high resistance to cyclodiene insecticides. A lower degree of resistance to lindane (8.5-fold) was evident, and the LD_{50} values for DDT and malathion were also elevated by 2- to 3-fold. It is possible that the method used in propagating this colony since 1957, while attempting to increase its level of susceptibility to DDT, may have been responsible for selection of a cyclodiene-resistant population. This possibility is being investigated in the laboratory.

The NAIDM (National Association of Insecticide and Disinfectant Manufacturers) strain was obtained from the Shell Development Corporation, Modesto, California, and shows a high degree of susceptibility to all common insecticides.

Three multiresistant strains (R-Super Pollard, R-Stauffer Chlorthion and R-Gardena) were used in the determination of cross-tolerances to carbamate insecticides. The R-Super Pollard strain has been maintained in this laboratory for a number of years under constant selection pressure with 50μg. DDT and 50μg. lindane per fly (March 1959). It is extremely resistant to DDT and analogues and to lindane and cyclodiene derivatives, but susceptible to organophosphorus insecticides. The R-Stauffer Chlorthion strain has been under Chlorthion® (*O*-(3-chloro-4-nitrophenyl)*O,O*-dimethyl phosphorothioate) selection pressure since 1955. It is resistant to Chlorthion and to other organophosphorus insecticides in various degrees and also to DDT and analogues, to lindane and to cyclodiene derivatives (March 1959). The R-Gardena strain was field collected at Gardena Dairy, Gardena, California, in November 1959. It is resistant to chlorinated hydrocarbon and cyclodiene insecticides and moderately tolerant to malathion and Chlorthion.

Selection Technique.—Rearing of the flies used in this study was carried out at 80° F. and 65% relative humidity, as previously described (March & Metcalf 1949). Both larval and adult pressure was employed in the selection of the strains, except for the strain (multiresistant to Compound III plus piperonyl butoxide) R-MIP+p.b. which was selected by larval pressure alone. The total selection pressure sought per generation was 90% to 95%. Each generation was begun usually with 10,000 or more eggs, and some 400 to 1,000 surviving flies constituted the parents of the succeeding generation.

Larval Pressure.—For the purposes of larval pressure, Compound III was dissolved in xylene (1:5 ratio) and a concentrated emulsion was prepared with Triton X-100 and water. Isolan was used in acetone solution because of its higher water solubility (David *et al.* 1960). In all instances, the required amount of insecticide was added to the rearing medium, after diamalt, brewer's yeast and water had been placed in the breeding jar and before addition of the alfalfa meal and wheat bran. The medium was thoroughly mixed during this operation to ensure uniform distribution of the toxicant. Records were kept of the approximate number of eggs used per jar (estimated on the basis of 2,000 eggs per 0.4 ml. in a graduated pipette) so that the percentage of adult emergence could be calculated.

Larval tolerance to xylene and Triton X-100 was investigated by incorporating these materials at various concentrations in the rearing medium. A significant reduction of adult emergence occurred when more than 8 ml. containing 20% xylene and 0.2% Triton X-100 in water were used per breeding jar. Throughout the larval selections with Compound III, the quantity of xylene and Triton X-100 used per jar did not exceed 6 ml. of this formulation.

Although the treatment of the rearing media is referred to as "larval" pressure, it should be mentioned that eggs were also affected by the treatment. However, the toxicant had no lethal effect on the pupae.

Adult Pressure.—All adult flies emerging from the treated media were collected each morning into holding cages provided with 20% sucrose solution and were treated on the nota within 8 hours thereafter by topical application of 1-microliter drops of standard w/v acetone solutions of the insecticides. Treatment before mating had occurred ensured that both male and female parents had been subjected to selection pressure. Feeding before treatment was considered desirable since treated flies remained in a state of paralysis for at least 8 hours thereafter, and consequently some would have been as much as 40 hours old before they had access to food. Treated flies of each generation were placed in a separate colony cage. A small number (50 to 100) were kept separately for 24 hours to provide information on the extent of mortality obtained. The degree of adult pressure sought depended on the apparent reduction obtained by larval pressure. If the latter was severe, adult pressure was somewhat relaxed, and vice versa.

Dosage-Mortality Regression Lines.—These were determined by topical application of 1-microliter drops of standard w/v acetone solutions of the insecticides to the nota of 3-day-old female flies reared in nontreated media (March & Metcalf 1949). Twenty flies were treated with each concentration and each treatment was replicated on at least three different dates. For the establishment of initial and final dosage-mortality curves of strains under pressure, treatments were replicated 6 to 10 times. The average percentage mortality obtained at each concentration was plotted on log-probit paper, and the dosage-mortality regression curves were fitted by eye. Treated flies were held for 24 ± 2 hours at 60° F. in 1-pint ice cream cartons with screen-wire ends and disposable paper liners and were supplied with 20% sucrose solution. The criterion of mortality was the inability to walk at least one full body length. No mortality resulted in control flies exposed to CO_2 anesthesia and acetone application. The standard test solutions were refrigerated at 0° C. when not in use.

RESULTS AND DISCUSSION.—*Selection of S-*Lab. *Strain with Compound III.—Development of R-*MIP *Strain.*—These selections were carried out for 22 generations. The larval, adult and total selection pressures were at an aver-

age level of 78%, 75% and 95% mortality, respectively. A different concentration of insecticide was used in selecting the adult male and female flies because of the greater susceptibility of males. The concentrations used were such that the percentage of surviving flies of each sex was approximately equal.

Female flies of the fourth generation showed approximately a twofold increase in tolerance (fig. 1A and table 1). By the seventh gereration a considerable degree of resistance had been achieved; the maximum mortality produced by dosages of more than 10 μg. per female fly did not exceed 37%. With further selection, this percentage diminished steadily, reaching 8% by the 14th generation. Further selection to 22 generations did not result in complete immunity. The development of resistance in male flies was considerably slower (fig. 1B and table 1), the LD_{50} value having increased only 2.3- and 3.4-fold by the 14th and 22nd generations, respectively, as compared with >50-fold increase in the females. However, approximately 6% of the F_{22} males could survive topical dosages of 10 μg. and this fraction would likely increase with further selection.

Table 1.—Progress of selection of house fly strains with carbamate insecticides.

	24-HOUR TOPICAL LD_{50} IN μG./FLY		
	Compound III		Isolan
GENERATION	Male	Female	Female
P_1	1.08	2.0	1.55
F_4	—	3.5	2.45
F_7	—	>100	—
F_9	—	—	5.9
F_{11}	—	—	7.4
F_{14}	2.48	>100	9.0
F_{20}	—	—	10.8
F_{22}	3.7	>100	—
Resistance ratio	3.4×	> 50×	7×

A comparison of larval susceptibility to Compound III (table 2) indicates a 7.5-fold resistance in the R_{MIP} strain. It will be observed that the ratio of male to female flies declined as the concentration of the toxicant increased, indicating that the greater susceptibility of males is also present in the larval stage. Limited tests on pupae by topical application indicated that the toxicant is of very low efficiency against this stage, 42% and 11% mortality having been obtained with 100 μg. per pupa of the $S_{Lab.}$ and R_{MIP} strains, respectively.

An examination of the dosage-mortality regression lines (fig. 1A, B) indicates that in most cases mortality was not increased with dosages greater than 10 μg. per fly. Application of higher concentrations apparently did not increase the rate of penetration of the toxicant through the cuticula and consequently the defense mechanism was able to prevent the accumulation of a lethal concentration at the site of action. A similar situation was observed with Compounds II and IV. Greater mortality was possible only by increasing the amount of solvent, thus increasing the area of the absorbing surface. It has already been pointed out that when very high dosages of

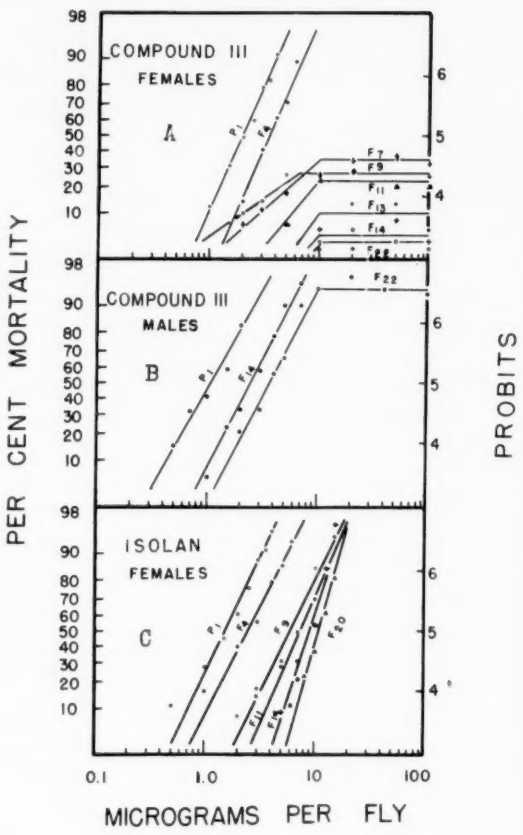

FIG. 1.—Dosage-mortality regression lines indicating development of resistance in house flies under carbamate-selection pressure. R_{MIP} strain: (A) females, (B) males; R_{Isolan} strain, (C) females.

Table 2.—Susceptibility of immature stages of $S_{Lab.}$ and R_{MIP} strains of house flies to Compound III as determined by adult emergence from treated media.[a]

DOSAGE IN WET WEIGHT OF MEDIA (P.P.M.)	PER CENT ADULT EMERGENCE		SEX RATIO
	$S_{Lab.}$	$R_{MIP(F_{21})}$	Male:Female
0	100		1.1:1
1,000	77		1.0:1
2,000	50		1.0:1
3,000	33		.9:1
0		100	1.1:1
20,000		16	1.1:1
23,000		7	.8:1
LD_{50} Resistance ratio	2,000 p.p.m.	15,000 p.p.m. 7.5×	

[a] No significant pupal or adult mortality due to treatment.

the toxicant are used, the increase in the amount penetrating through the cuticula is not proportional to the increase of the dose applied (Perry 1952, Winteringham 1952, Tahori & Hoskins 1953, Barker & Rawhy 1957). The present data indicate that the mortality obtained with 100 μg. of Compound III was, in most cases, even lower than that obtained with 20 μg. or 50 μg. (fig. 1A, B). This is attributed in part to faster crystallization of the toxicant when used at such high concentrations, so that the area covered by the solvent is smaller.

Because of the limitations in absorption of Compound III at dosages greater than approximately 10 μg. per fly, the establishment of straight dosage-mortality regression lines for the R- strains has not been possible and consequently, the measurement of the increase in resistance in terms of the LD_{50} could not be accomplished. As an alternative, the toxicant was dissolved in dioctyl phthalate and LD_{50} values were determined by topical application and injection (table 3 and fig. 2). Use of dioctyl phthalate alone produced no mortality or visible abnormality in the test insects. The insecticide was less toxic to the S- flies when it was applied topically in dioctyl phthalate solution instead of in acetone. On the R- flies, however, higher mortality was obtained with the insecticide in dioctyl phthalate solution. As a result, the degree of resistance as determined by topical application using dioctyl phthalate was considerably lower, *i.e.*, 7-fold, as compared with >50-fold when acetone was used.

The foregoing data are in agreement with published results on the use of DDT in acetone and oil (Barker & Rawhy 1957, Oppenoorth & Dresden 1958). Use of an oil as solvent at these high concentrations produces greater absorption per unit of time as a result of the spread of the solvent over a wider area of body surface. Injection of the insecticide results in the instantaneous introduction of toxicant at a much higher concentration within the body tissues than is obtained when the same dose is applied externally. The results shown in table 3 indicate that the LD_{50} value for injected Compound III on the R_{MIP} strain was >23 and 5.1 times as low as those obtained by topical application in acetone and dioctyl phthalate, respectively.

Selection of R_{MIP} Strain with Compound III Plus Piperonyl Butoxide (p.b.).—Development of $R_{MIP+p.b.}$ Strain.—Examination of the cross-tolerances of R_{MIP} after 11 generations of selection indicated that although the strain was highly resistant to Compound III, it exhibited only a twofold cross-tolerance to the combination of Compound III and piperonyl butoxide (table 4). Selection of a subcolony of R_{MIP} with Compound III plus piperonyl butoxide was undertaken with the expec-

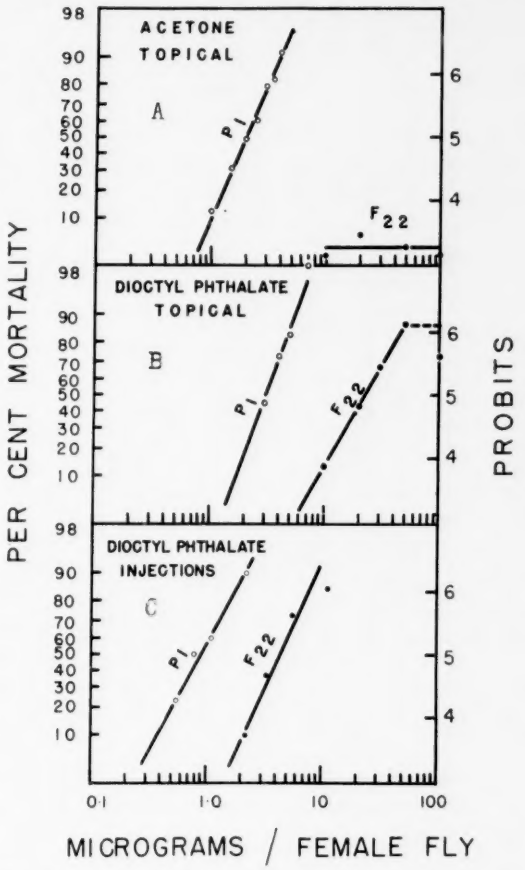

FIG. 2.—Response of $S_{Lab.}$ and R_{MIP} strains of house flies to Compound III: (A) topical in acetone, (B) topical in dioctyl phthalate, (C) injections.

Table 3.—Influence of method of application on the observed toxicity of Compound III (3-isopropylphenyl *N*-methylcarbamate) against $S_{Lab.}$ and R_{MIP} strains of house flies.

Method of Application	Solvent	Volume (μL.)	Site	24-Hour LD_{50} in μg./Female Fly		Resistance Ratio
				$S_{Lab.}$	R_{MIP}	
Topical	Acetone	1	Notum	.2	>100	>50×
Topical	Dioctyl phthalate	1	Notum	3.2	22	7×
Injection	Dioctyl phthalate	.112	Pronotum	.88	4.3	4.9×

Table 4.—Progress of selection of R_{MIP} (F_{11}) strain of house flies with a combination of Compound III and piperonyl butoxide (p.b.) (1:5 ratio) by larval pressure in the laboratory.

Generation	Larval Treatment in Wet Weight of Media (p.p.m.)		Selection Pressure (%)	Topical LD_{50} of Adults (μg./female fly)		
	Compound III	P.b.		Compound III	P.b.	Resistance ratio
$S_{Lab.}$				0.33	1.65	—
$R_{MIP}(F_{11})$ (P1)				.68	3.40	2×
F1	6,000	30,000	91			
F2	7,000	35,000	98			
F3	8,000	40,000	95			
F4	10,000	50,000	99			
F5	10,000	50,000	98			
F6	10,000	50,000	93			
F7	10,000	50,000	92			
F8	10,000	50,000	89	1.00	5.00	3×
F9	10,000	50,000	92			
F10	10,000	50,000	90			

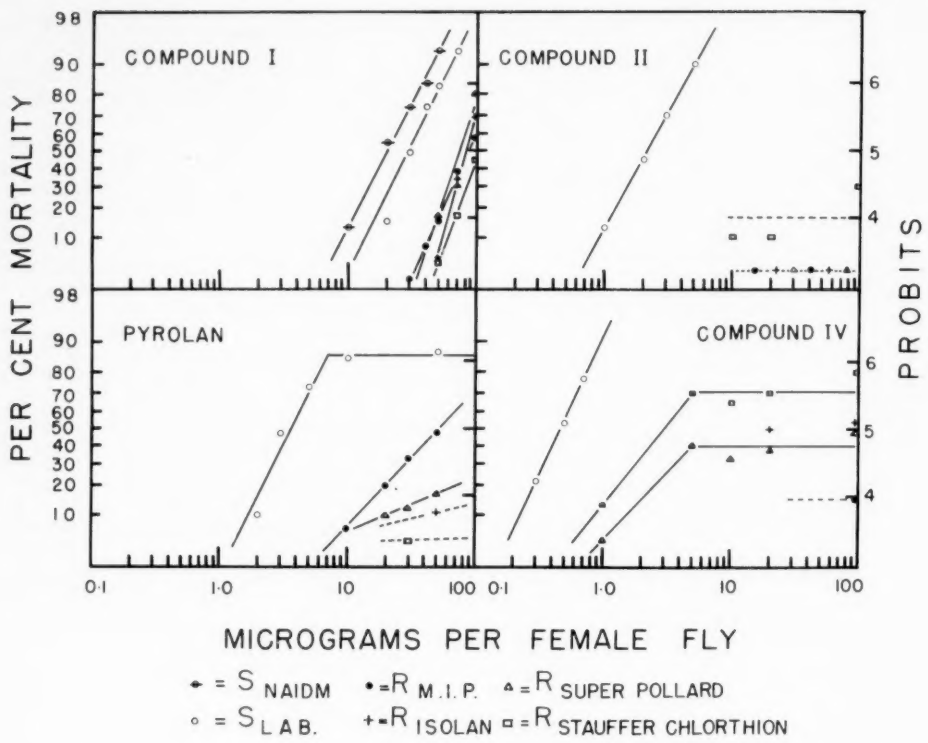

FIG. 3.—Cross-resistance to carbamate insecticides in carbamate-selected and multiresistant strains of house flies.

tation that the potentialities for development of resistance to this combination would be expressed in a short period of time since the strain was already resistant to the insecticide (March *et al.* 1953). Selection was confined to the larval stage and was at an average level of 94% mortality (table 4). Larval tolerance rose from 6,000 p.p.m. to about 10,000 p.p.m. of synergized Compound III during the first four generations. Beyond that the increase in tolerance was very limited as evidenced by the degree of pressure achieved on succeeding generations which were raised on media containing the same concentration of insecticide. Tolerance to Compound III plus piperonyl butoxide in F_8 adult flies was only 1.5-fold as great as in the parental strain ($R\text{-}_{MIP\text{-}F_{11}}$) and 3-fold greater than in the $S\text{-}_{Lab.}$ strain (table 4). Although continuation of selection with the combination of carbamate and synergist may result in a further small increase in tolerance, the results of the rigorous although limited selection reported here, and Moorefield's (1960) less intense but prolonged selection with synergized Sevin, suggest the likelihood of biochemical limitations in the ability of house flies to develop a defense mechanism of high efficiency against the combination of carbamate and piperonyl butoxide.

Selection of $S\text{-}_{Lab.}$ Strain with Isolan.—*Development of $R\text{-}_{Isolan}$ Strain.*—Selection with Isolan progressed at a somewhat slower rate than with Compound III, because of sluggishness and delayed oviposition in the treated insects. Flies emerging from treated media, but not treated in the adult stage, behaved normally and mated and laid eggs promptly. Twenty generations of selection pressure were completed. Larval pressure was applied in all generations, but adult pressure was omitted in four cases because of either high larval mortality or of the need for increasing the size of the colony for dosage-mortality tests. The larval, adult and total selection pressures exerted on all 20 generations were at an average level of 78%, 67% and 93% mortality, respectively. Adult tolerance to Isolan rose gradually from an LD_{50} of 1.55 μg. per female fly for the susceptible strain to 10.8 μg. for the 20th generation of the selected strain, an increase of 7-fold (table 1 and fig. 1C). During the same period larval tolerance had increased 19.5-fold (table 5).

Cross-Resistance in Carbamate-Selected Strains.—These tests were performed on generations F_{18-22} of $R\text{-}_{MIP}$ and F_{18-20} of $R\text{-}_{Isolan}$ when both strains were believed to have attained near maximal resistance to the selecting agents. Data on $R\text{-}_{Super\ Pollard}$ and $R\text{-}_{Stauffer\ Chlorthion}$ have been included for comparison (figs. 3 and 4). The highest dose employed was 100 μg. When the mortality obtained did

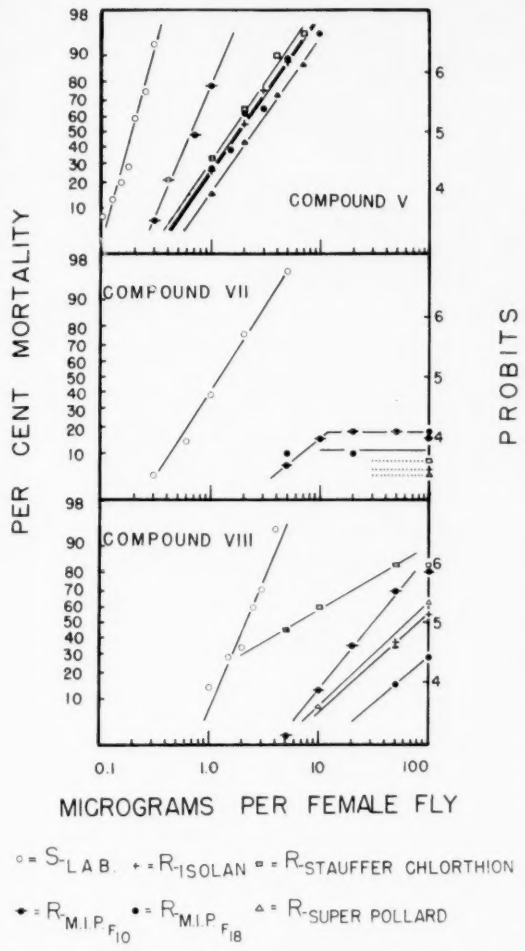

FIG. 4.—Cross-resistance to carbamate insecticides in carbamate-selected and multiresistant strains of house flies.

Table 5.—Susceptibility of immature stages of S-Lab. and R-Isolan strains of house flies to Isolan, as determined by adult emergence from treated media.[a]

ISOLAN IN WET WEIGHT OF MEDIA (P.P.M.)	PER CENT ADULT EMERGENCE		SEX RATIO
	S-Lab.	R-Isolan(F19)	Male:Female
0	100		1.1:1
200	60		.9:1
300	18		.8:1
400	11		.5:1
0		100	1.2:1
3,000		95	1.0:1
4,000		72	.7:1
5,000		17	.5:1
LD_{50} Resistance ratio	220 p.p.m.	4,300 p.p.m. 19.5×	

[a] No significant pupal or adult mortality due to treatment.

not exceed 50%, the LD_{50} is indicated as >100 μg. The calculated values for "resistance ratio" represent the product

$$\frac{LD_{50}\ R\text{- strain}}{LD_{50}\ S\text{- strain}}.$$

Group 1: Carbamates.—Selections by Moorefield (1960) with Compound III produced cross-tolerance to Isolan and Pyrolan of 1.6- and 1.7-fold, respectively. Selection of a Diazinon-resistant strain with Pyrolan produced cross-tolerance to Compound III and Isolan of 2- and 3-fold, respectively.

The results obtained (table 6) in the present study indicate that selection with Compound III or Isolan produced high levels of cross-tolerance or cross-resistance to all nine carbamates tested. Both R- strains were virtually immune to the substituted phenyl *N*-methyl carbamates: Compound II (2-isopropyl-), Compound III (3-isopropyl-), Compound IV (2-isopropoxy-), and Compound VI (3-*tert*-butyl-), and to Sevin. Notable exception among the carbamates in this class was Compound V (3,5-dimethoxy-), which continued to exhibit some degree of toxicity toward the selected strains, the LD_{50} values for R_{Isolan} and R_{MIP} showing increases of only 9.2- and 9.7-fold, respectively. At the LD_{95} level, however, the increase in resistance was near 50-fold, and there can be little doubt that with additional selection further resistance to this carbamate will also be produced.

Compound I (phenyl *N*,*N*-dimethylcarbamate) behaved differently from the *N*-methyl carbamates in that, in spite of its very low efficiency against the S- strains, it retained a small degree of toxicity against all R- strains (fig. 3). The LD_{50} values of R_{MIP} and R_{Isolan} with this compound showed an increase of only 2.8- and 2.6-fold, respectively. Of the two pyrazolyl *N*,*N*-dimethyl carbamates, Pyrolan was almost ineffective against the R- strains, and it also exhibited poor performance against the $S_{Lab.}$ strain. Isolan, on the contrary, continued to be relatively active against both R- strains. The R_{MIP} strain showed a 5.5-fold cross-resistance to this compound as compared with a 7-fold resistance exhibited by the R_{Isolan} strain.

Cross-tolerance to the combination of carbamate and piperonyl butoxide increased much less than to the carbamate alone. It will be observed (table 6) that while resistance to Isolan in the R_{Isolan} strain increased by 7-fold, cross-tolerance to Isolan plus piperonyl butoxide increased by only 4.5-fold. Similarly, cross-tolerance to Compound III plus piperonyl butoxide in R_{MIP} increased by only 2.7-fold as compared with an undeterminable degree of resistance toward Compound III alone.

Group 2: DDT and Methoxychlor.—Both R- strains showed only slight increase in cross-tolerance to DDT (1.2- and 1.3-fold); they exhibited, however, some cross-resistance to methoxychlor (5.1- and 5.2-fold) (see table 6). This is not surprising since it is known that resistance to methoxychlor is not always accompanied by resistance to DDT (Barber & Schmitt 1949, Bruce & Decker 1950). Neither Meltzer (1956) nor Moorefield (1960) has reported significant cross-tolerance to DDT at the LD_{50} level in carbamate-selected strains.

Table 6.—Response of susceptible and laboratory-selected carbamate-resistant strains of house flies to topical application of various insecticides.

Group	Insecticide	24-Hour Topical LD_{50} in μg./Female Fly		
		S-Lab. (P_1)	R-MIP (F_{18-22})	R-Isolan (F_{18-20})
1	Isolan	1.55	8.5	10.8
	Pyrolan	3.7	55	>100
	Sevin	>100	>100	>100
	Compound I (phenyl *N*,*N*-dimethylcarbamate)	31	88	82
	II (2-isopropylphenyl *N*-methylcarbamate)	2.15	>100	>100
	III (3-isopropylphenyl *N*-methylcarbamate)	2	>100	>100
	IV (2-isopropoxyphenyl *N*-methylcarbamate)	.47	>100	20
	V (3,5-dimethoxyphenyl *N*-methylcarbamate)	.19	1.85	1.74
	VI 3-*tert*-butylphenyl *N*-methylcarbamate)	>100	>100	>100
	Isolan plus piperonyl butoxide	.175	.75	.78
	Compound III plus piperonyl butoxide	.33	.88	.73
2	DDT	.09	.1	.13
	Methoxychlor	.1	.69	.7
3	Lindane	.085	>100	>100
	Aldrin, dieldrin, heptachlor, toxaphene	>100	>100	>100
4	Prolan	.08	.16	.2
5	Parathion	.02	.08	.06
	Malathion	1.47	6.4	6.1
	Chlorthion	.72	.8	1.24
	Diazinon	.07	.38	.31
	Dicapthon	.05	.08	.1
	Ronnel	.084	.45	.42
6	Allethrin	.25	.39	1.05

Group 3: *Lindane and Cyclodiene Derivatives.*—Since the parental (S-Lab.) strain possessed considerable resistance to the cyclodiene derivatives, no conclusion can be drawn regarding the effect of the present selections on cross-resistance to these compounds. The increase in the LD_{50} value for lindane from 0.085 μg. to 100 μg. (table 6) may have resulted from the stress brought upon the population by the severity of the selection pressure as such and not to the specificity of the selecting agent. This resulted in completion of the resistance spectrum toward the compounds in this category. The question is complicated, however, by the high cross-resistance levels toward the cyclodiene derivatives exhibited by Meltzer's (1956) Compound I-selected strain. This matter deserves further consideration.

Group 4: *Nitroparaffins.*—Tests with Prolan indicated a 2- and 2.4-fold level of cross-tolerance to this compound in the R-MIP and R-Isolan strains, respectively (table 6).

Group 5: *Organophosphorus Insecticides.*—Information on cross-resistance to organophosphorus insecticides in carbamate-selected strains is available only for Diazinon. Moorefield (1960) found no cross-tolerance to this compound in a strain maintained under Sevin pressure for 11 generations. The period of selection pressure may have been too short and the selecting agent of too low efficiency to have induced full expression of the tolerance potential. Meltzer's (1956) data indicate a sixfold increase in the LD_{50} level for Diazinon in the strain selected with Compound I. Finally, Wiesmann (1956) reported that a Pyrolan-selected strain was cross-resistant to Diazinon. The degree of cross-resistance is not known.

Both carbamate-resistant strains developed in this work exhibited cross-tolerance to organophosphorus insecticides of less than approximately fivefold (table 6). The compounds may be classified on the basis of the cross-tolerance ratios as ronnel > Diazinon > malathion > parathion > dicapthon > Chlorthion. The cross-tolerance levels are not correlated to the initial LD_{50} values of the respective compounds on the S-Lab. strain. It will be observed that both R- strains exhibited approximately the same degree of cross-tolerance toward each organophosphorus insecticide tested. This may suggest a similarity in the cross-tolerance mechanism toward organophosphorus insecticides in these two strains.

Group 6: *Pyrethroids.*—Data with allethrin indicate 1.5- and 4.2-fold increases in the LD_{50}'s of the R-MIP and R-Isolan strains, respectively.

Cross-resistance to Carbamates in Noncarbamate-Selected Strains.—Several workers have presented data on the susceptibility of laboratory-selected chlorinated hydrocarbon-resistant and organophosphorus insecticide-resistant strains to carbamate insecticides (Meltzer 1956, Wiesmann 1956, Eldefrawi *et al.* 1959, 1960, LaBrecque *et al.* 1959, Forgash & Hansens 1959, 1960, Moorefield 1960). These studies contain interesting information but are usually limited to a small number of carbamates. The results obtained in this study with R-Super Pollard and R-Stauffer Chlorthion (table 7, figs. 3 and 4) may serve to illustrate the following characteristics of R- strains:

(a) Strains which have attained maximum levels of resistance to chlorinated hydrocarbon or organophos-

Table 7.—Response of multiresistant strains of house flies to carbamate insecticides.

INSECTICIDE	24-HOUR TOPICAL LD_{50} IN μG./FEMALE FLY			
	S-Lab.	R-Super Pollard	R-Stauffer Chlor.	R-Gardena
Isolan	1.55	8.8	9.9	4.7
Pyrolan	3.7	>100	>100	
Sevin	>100	>100	>100	
Compound I (phenyl *N*,*N*-dimethylcarbamate)	31	66	>100	
II (2-isopropylphenyl *N*-methylcarbamate)	2.15	>100	>100	
III (3-isopropylphenyl *N*-methylcarbamate)	2	20	8.3	3.7
IV (2-isopropoxyphenyl *N*-methylcarbamate)	0.47	>100	3	
V (3,5-dimethoxyphenyl *N*-methylcarbamate)	0.19	2.5	1.43	
VI (3-*tert*-butylphenyl *N*-methylcarbamate	>100	>100	>100	
Isolan plus piperonyl butoxide	0.175	1.0	1.04	
Compound III plus piperonyl butoxide	0.33	0.86	0.63	

phorus insecticides, or to both, show varying degrees of cross-tolerance or cross-resistance to carbamate insecticides. Cross-resistance to the *N*,*N*-dimethyl carbamates Isolan, Dimetilan® (3-methyl-5-pyrazolyl dimethylcarbamate) and Compound I is relatively low. An exception to the carbamates in this category is made by Pyrolan which was ineffective against the R- strains. Cross-resistance to the *N*-methyl carbamates, Compounds II, III, IV, V, VI and Sevin, is higher; and although the resistance ratios in some instances appear low, the dosage-mortality lines obtained are usually broken or flat, indicating that a segment of the population is immune to the compound, as determined by the topical method.

(b) Information concerning differences between the degree of cross-resistance in R-Chlorinated hydrocarbon and R-Organophosphorus strains toward a given carbamate is conflicting. LaBrecque *et al.* (1959) reported identical cross-resistance ratios toward two carbamates in two R- strains, although one was decidedly more resistant to DDT and the other more resistant to malathion. Moorefield (1960) found that Isolan and Compound III were approximately as toxic to an R-DDT as to an R-Diazinon strain. However, data by Eldefrawi *et al.* (1959, 1960) show greater cross-resistance to Sevin, Dimetilan, Isolan and Pyrolan in an R-parathion than in an R-DDT strain. The present data (table 7) indicate greater cross-resistance to *N*-methyl carbamates (Compounds II, III, IV and V) in the chlorinated hydrocarbon-selected strain (R-Super Pollard) and greater cross-resistance to *N*,*N*-dimethyl carbamates (Isolan, Pyrolan and Compound I) in the organophosphorus-selected strain (R-Stauffer Chlorthion). Whether these differences are caused by the type of selection agent used in the development of these strains, or to chance inclusion of the character in the genetic makeup of the parental strains, may become clearer as data from additional R-strains become available.

CONCLUSIONS.—*Resistance Potential.*—Data on synergism of carbamates with 3,4-methylenedioxyphenyl compounds (Moorefield 1958) indicate the presence of a mechanism for enzymatic detoxication of carbamates in house flies. Since this mechanism is generally present in nonselected populations, it may be considered as natural tolerance. Available data on the activation ratio of carbamates with piperonyl butoxide (Metcalf *et al.* 1960) indicate that the degree of this tolerance varies with the particular carbamate and is inversely correlated with the innate toxicity of the compound. Since variation is characteristic of natural populations, it may be expected that selection pressure with a carbamate will result in intensification of this tolerance. This has been amply demonstrated in the present study by the production of carbamate-resistant strains through selection with Compound III and Isolan.

The potentialities of house flies for development of resistance to carbamates are also evident in the multiresistant R-Super Pollard, R-Stauffer Chlorthion and R-Gardena strains. Although none of these has a history of exposure to carbamates, they all show varying degrees of cross-tolerance or cross-resistance to these compounds (see table 7).

Resistance Levels.—It has been suggested that the degree of resistance attainable toward a given carbamate depends on the susceptibility level of the original strain toward this compound (Moorefield 1960). While some relationship may exist between initial toxicity, slope of the dosage-mortality curve and possible degree of resistance, this does not always appear to be the case. Resistance to Compound I (LD_{50} 31) remained low (2- to 3.2-fold), while resistance to Compound IV and Compound V (LD_{50} 0.47 and 0.19, respectively) is considerably more elevated (see table 6).

High levels of resistance toward all *N*-methyl carbamates tested (Compounds II, III, IV, V, and VI, and Sevin) were present in the selected strains. The degree of resistance toward these compounds appeared to be magnified by absorption limitations when doses in excess of about 10 μg. per fly were employed in topical application. Injection of Compound III reduced the resistance ratio from >50-fold to 4.7-fold. These results reflect the differences in rates of penetration and accumulation of the toxicant in critical concentrations at the sites of action.

Resistance to the *N*,*N*-dimethyl carbamates Isolan and Compound I remained at a relatively low level (7- and 2.8-fold, respectively) in spite of intensive selection. There was no evidence of limitations in absorption of these compounds. Cross-resistance to the *N*,*N*-dimethyl carbamate Pyrolan was elevated, and limitations in absorption were evident.

When carbamates were used with piperonyl butoxide, the resistance ratios obtained were less than sixfold. Further selection with Compound III plus piperonyl butoxide resulted in only slight increase in the tolerance level to

this combination. These results indicate that physiological resistance to the combination of carbamate and piperonyl butoxide may be limited, although maximal levels of resistance to the carbamate alone may be reached which appear elevated as a result of limitations in absorption rates.

Cross-Tolerances and Specificity of Resistance.—Both carbamate-selected strains showed relatively low cross-tolerance levels (<3-fold) to DDT, Prolan, parathion, Chlorthion and dicapthon, and higher cross-tolerance or low cross-resistance (3- to 6-fold) to methoxychlor, malathion, Diazinon, ronnel and allethrin. In this respect, the carbamate-selected strains differ from organophosphorus-selected strains in that the latter show high cross-resistance to DDT and related compounds.

Cross-resistance tests also indicate that selection with a carbamate results in varying degrees of resistance to carbamates belonging to distinctly different groups. However, a certain degree of specificity, as far as the selection agent and related compounds are concerned, is evident. For instance resistance to either Compound III or to Isolan is higher in the strain selected with the particular compound. Similarly, the Isolan-selected strain shows greater cross-resistance to the related compound Pyrolan, while the Compound III-selected strain shows greater cross-resistance to the related Compound IV.

Resistance to carbamates may, therefore, consist of an intensified broad-defense mechanism occurring in natural populations, plus a more discriminating mechanism (or mechanisms) particular to the selecting agent and to closely related compounds. The present work suggests some degree of specificity within the *N*-methyl and *N,N*-dimethyl carbamate groups. Work by Metcalf *et al.* (1960) indicates that detoxication mechanisms may further discriminate between such closely related compounds as 3,4- and 3,5-dimethoxyphenyl *N*-methylcarbamates.

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Evaluation of Chemical and Microbial Materials for Control of the Cabbage Looper[1]

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Abstract

A number of chemical insecticides and some formulations of commercially produced microbial preparations containing *Bacillus thuringiensis* var. *thuringiensis* Berliner were applied as sprays and/or dusts to cabbage, cauliflower, and head lettuce for control of the cabbage looper, *Trichoplusia ni* (Hbn.). Only one of the chemical materials, General Chemicals 3583® (diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate) appeared to be comparable to the standards, DDT-toxaphene and parathion. However, several insecticides, including Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), ethion, Guthion® (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3 (4*H*)-ylmethl phosphorodithioate), malathion, Perthane® (1,1-dichloro-2,2-bis (*p*-ethylphenyl)ethane), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide), and Sevin® (1-naphthyl methylcarbamate) gave fair results. The microbial materials from different manufacturers varied in their effectiveness. Two gave good control of all sizes of cabbage loopers and displayed satisfactory residual activity. At the dosages used, they were as effective in quickness of kill and residual activity as the best of the chemical insecticides.

The problem of maintaining effective control of the cabbage looper, *Trichoplusia ni* (Hbn.), on the many crops on which it is a pest in California has reached serious proportions in recent years as field populations of the insect have become more difficult to control with the commonly used insecticides. At present there is no one chemical that will provide consistently good control of the cabbage looper. The probability of increased resistance of the looper to DDT has been recognized in New York (McEwen & Hervey 1956), Texas (Parencia *et al.* 1957), and Ontario and Quebec, Canada (Harcourt & Cass 1959). The problem is intensified further by residue restrictions, which reduce the number of insecticides that can be used as the harvest date is approached. These difficulties have necessitated an intensive evaluation of both currently used and experimental materials in order to select the most suitable insecticides for incorporation into recommended treatment programs.

In past years the only materials available for control of the cabbage looper were chemical insecticides. However, interest in the use of insect pathogens as an alternative to chemical control has followed the reports by Tanada (1956) and Hall & Dunn (1958) on the susceptibility of the pest to the crystalliferous spore-forming bacterium, *Bacillus thuringiensis* var. *thuringiensis* Berliner. The subsequent commercial mass culture of the bacillus and wide-scale distribution by several manufacturers of improved experimental insecticides containing the insect pathogen have permitted broader field testing. Partial or effective control of the cabbage looper with the use of the new microbial materials has been reported by a number of workers. McEwen & Hervey (1959) obtained satisfactory control of the insect on broccoli by using sprays of the bacillus at dosages equivalent to 1 pound or more per acre of a spore preparation containing 100 billion spores per gram. Using the same arbitrary spores-per-gram basis for the purpose of standardization, Hall & Andres (1959) recognized that the dosage of the microorganism most suitable for control of the cabbage looper will vary with the field population, larval size, and plant size. They suggested a dosage range of about 0.5 to 1.5 pounds of 100 billion spores per gram material for effective control of the pest on crucifers.

The purpose of the study on which this report is based was to evaluate various currently used and experimental insecticides, incorporating both chemical materials and commercial formulations of *Bacillus thuringiensis* var. *thuringiensis*, for control of populations of the cabbage looper on crucifers and lettuce in California.

Materials, Methods, and Results.—The chemical insecticides used in the tests were DDT, toxaphene, parathion, malathion, ethion, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate), Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), dimethoate, Guthion® (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3 (4*H*)-ylmethyl phosphorodithioate), Perthane® (1,1-dichloro-2,2-bis(*p*-ethylphenyl) ethane), Sevin® (1-naphthyl methylcarbamate), Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9,-methano-2,4,3-benzodioxathiepin 3-oxide), General Chemicals 3583 (diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate), and Silica Gel 78.

The microbial insecticides containing *Bacillus thuringiensis* var. *thuringiensis* were obtained from Bioferm Corporation, Wasco, California; Merck & Co., Inc., Rahway, New Jersey; Nutrilite Products, Inc., Buena Park, California; Rohm and Haas Company, Philadelphia, Pa., and Stauffer Chemical Company, New York, New York. The first bacillus materials that were evaluated were the initial products of industrial development; more advanced insecticidal preparations that were received from the manufacturers were used in the last three tests. Although none of the microbial products were furnished with indices of activity to denote accurate levels of toxicity to insects, the manufacturers supplied their determinations of virulence based on the number of viable spores per gram of dry material as a guide to the potency of each preparation. Since each microbial product was considered to be an insecticidal compound stabilized as to spore count and toxicity by the manufacturer, no attempt was made to obtain additional rates of activity prior to use in the field tests other than to ascertain pathogenicity against a standard laboratory bioassay insect, the salt-marsh caterpillar, *Estigmene acrea* (Drury).

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All the experiments were designed as randomized complete blocks with four replicates. The materials in the first two tests were applied with hand equipment. The sprays were put on using two or there TeeJet hollow-cone nozzles per row at 40 pounds pressure. Dust treatments were made with a rotary-type hand duster with a single outlet, and both sides of each row were dusted separately to obtain good coverage. Unless otherwise noted, application of materials in the remaining tests was made with ground power equipment. Sprays were applied with a tractor-drawn Hardie rig which operated at 100 p.s.i. Two TeeJet No. 4 or No. 6 hollow-cone nozzles were used per row. The dusts were applied with a tractor-mounted Niagara duster using one outlet per row.

Sampling of the insect populations was accomplished at intervals after treatment by the examination of randomly selected plants in each plot and the recording of the number of living small (first instar), medium (second instar to ½ inch in length), and large (over ½ inch in length) cabbage loopers.

Test No. 1.—Ten standard and experimental chemical insecticides were sprayed and/or dusted on young cabbage at Riverside, California, on October 13, 1958. The sprays were applied at the rate of 40 to 50 gallons per acre. Each plot consisted of two 2-row beds 50 feet in length. The cabbage plants were 6 to 8 inches high. The results of this test are presented in table 1.

Table 1.—Evaluation of chemical insecticides against cabbage loopers on cabbage. Riverside, California. October 1958.

Treatment	Actual Toxicant /Acre (lb.)	Mean No. Loopers/100 Plants[1] 3 Days		
		Small	Medium	Large
Sprays				
Gen. Chem. 3583	0.6	0^{a}	2^{a}	2^{a}
Dibrom	1.0	0^{a}	7^{cd}	6^{abcd}
Parathion-Perthane	1.0–1.0	0^{a}	3^{ab}	4^{ab}
Malathion-Perthane	1.2–1.2	0^{a}	8^{cd}	5^{abc}
Parathion	1.2	0^{a}	3^{ab}	3^{ab}
DDT-toxaphene	2.0–3.0	1^{a}	4^{abc}	2^{a}
Dusts				
Thiodan, 3%	1.1	0^{a}	13^{de}	17^{de}
Sevin, 10%	1.9	1^{a}	12^{cd}	7^{abcd}
Parathion, 2%	0.8	2^{a}	6^{bcd}	9^{bcd}
DDT, 10%-toxaphene, 15%	2.9| 4.4/	2^{a}	14^{de}	12^{cd}
Untreated		7^{b}	27^{e}	31^{e}

[1] a,b,c,d,e Any two means in the same column having the same letter for a superscript are not significantly different as determined by Duncan's multiple range test at the 5% level. Original data were transformed to logarithms for analysis.

Because only a single examination of 120 plants per treatment was made at the end of 3 days on October 16, the results are indicative of contact, but not residual, effectiveness of the materials. In addition, the low population of small larvae required comparisons among materials to be based largely on counts of medium and large loopers, which are considerably more difficult to kill than first-instar larvae. The better performance of the DDT-toxaphene mixture as a spray than as a dust may be due to the excellent coverage of the plants which was obtained by the use of three nozzles per row. All the materials applied as sprays seemed worthy of further testing when compared with DDT-toxaphene and parathion, which are regarded as standards by virtue of their fairly consistent performance in the field.

Test No. 2.—Five chemical-insecticide treatments and two dosage levels of a bacillus wettable powder were applied as sprays, and two concentrations of a bacillus dust formulation were dusted on 5- to 6-inch-high head lettuce at Ventura, California, on October 22, 1958. The sprays were applied at the rate of 36 to 41 gallons per acre. Plots were three 2-row beds 50 feet long. On October 24 and 27 and November 3, 48 plants per treatment were examined. The results of this test appear in table 2.

A constant reinfestation of the field produced a good test of the residual effectiveness of the materials used. Ignoring the counts of small larvae, which might include only recently hatched first-instar larvae that had not yet contacted sufficient toxicant to cause mortality, only parathion and DDT-toxaphene might be considered to have given effective control at 12 days after application. It is of interest that the addition of Perthane to parathion did not appear to produce a better degree of larval control than an approximately equal dosage of parathion alone.

The results with the microbial materials tend to support the reports by Hall & Andres (1959) and Grigarick & Tanada (1959) on the apparent superiority of dust over spray formulations of commercial preparations containing *B. thuringiensis*. Although the application of the highest dosage wettable powder caused a noticeable reduction after 5 days in the number of medium and large larvae, the level of control with the bacillus spray materials was relatively low. The bacillus dust preparations gave somewhat better results, with the lower concentration material being as effective at the end of 12 days as the DDT-toxaphene spray. The increased effectiveness of this preparation may be attributed to an improvement in characteristics of flowability and adherence brought about by a 50% dilution with pyrophyllite of the celite-based material that was actually released for use as a wettable powder.

Test No. 3.—Four chemical sprays and two low-concentration bacillus preparations, one a wettable powder and one a dust, were applied (the sprays at the rate of 50 gallons per acre) to 5- to 6-inch-high head lettuce with a light looper population near Oxnard, California, on April 13, 1959. The plots were four 2-row beds 370 feet long. On April 16, 60 plants per treatment were examined. The results are presented in table 3.

A general trend which has been observed in many chemical control studies of the cabbage looper may be noted here. DDT-toxaphene tends to be more effective than parathion against the larger larvae, but less effective against small larvae. General Chemicals 3583, which proved to be as good as or better than parathion and DDT-toxaphene in many tests, generally produces the same type of larval kill pattern as parathion.

This was the first test involving a microbial material formulated and released by the manufacturer as a wettable powder and a dust under a trade name. Good control of the light looper population was not attained with the dust even at the high rate of 51 pounds per acre.

Test No. 4.—A number of chemical materials and

Table 2.—Evaluation of chemical and microbial insecticides against cabbage loopers on lettuce. Ventura, California. October 1958.

Treatment	Actual Toxicant /Acre (lb.)[2]	Mean No. Loopers/100 Plants[1]								
		2 Days			5 Days			12 Days		
		Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
Sprays										
Gen. Chem. 3583	0.5	35^{ab}	15^{abc}	4^{a}	27^{c}	25^{bcd}	4^{a}	75^{ab}	48^{cd}	2^{a}
Parathion-Perthane	0.9–0.9	27^{a}	12^{a}	0^{a}	4^{a}	15^{ab}	6^{a}	46^{a}	4^{a}	8^{ab}
Parathion	1.0	19^{a}	13^{ab}	0^{a}	10^{bc}	4^{a}	0^{a}	44^{a}	6^{a}	0^{a}
Perthane	1.0	96^{cd}	31^{abc}	2^{a}	125^{ef}	65^{ef}	2^{a}	156^{d}	83^{de}	17^{b}
DDT-toxaphene	2.0–3.0	69^{bc}	19^{abc}	2^{a}	54^{d}	35^{cde}	0^{a}	44^{a}	29^{bc}	2^{a}
Bacillus (1.5×10^{10} spg)[3]	4.8	131^{cd}	21^{abc}	0^{a}	154^{ef}	40^{de}	0^{a}	131^{cd}	87^{de}	0^{a}
Bacillus (1.5×10^{10} spg)[3]	2.2	154^{cd}	52^{bc}	10^{a}	189^{f}	90^{f}	2^{a}	135^{d}	119^{de}	4^{a}
Dusts										
Bacillus (3×10^{9} spg)[4]	28	79^{bcd}	31^{abc}	2^{a}	90^{de}	19^{bc}	0^{a}	102^{bcd}	48^{cd}	2^{a}
Bacillus (1.5×10^{9} spg)[5]	28	75^{bc}	33^{abc}	2^{a}	58^{d}	19^{bc}	2^{a}	77^{bc}	21^{ab}	0^{a}
Untreated		192^{d}	50^{c}	6^{a}	196^{f}	90^{f}	8^{a}	162^{d}	158^{e}	27^{b}

[1] Same as in table 1.
[2] Poundage for bacillus represents actual amounts of formulated material used.
[3] Merck & Co., Inc., experimental microbial insecticide, Lot No. E-9745.
[4] Bioferm Corp., 57-18 microbial insecticide.
[5] Bioferm Corp., 57-18 microbial insecticide cut 50% with superpyrol.

Table 3.—Evaluation of chemical and microbial insecticides against cabbage loopers on lettuce. Oxnard, California. April 1959.

Treatment	Actual Toxicant /Acre (lb.)[2]	Mean No. Loopers/100 Plants[1]		
		3 Days		
		Small	Medium	Large
Sprays				
Gen. Chem. 3583	0.9	0^{a}	10^{b}	3^{a}
DDT-toxaphene	1.9–2.9	3^{ab}	2^{a}	0^{a}
Dimethoate	0.8	10^{ab}	35^{c}	5^{a}
Parathion	0.9	0^{a}	13^{b}	3^{a}
Bacillus (3×10^{9} spg)[3]	3.0	13^{ab}	31^{c}	5^{a}
Dusts				
Bacillus (3×10^{8} spg)[4]	51.0	12^{ab}	40^{c}	3^{a}
Untreated		17^{b}	26^{c}	2^{a}

[1] Same as in table 1.
[2] Same as in table 2.
[3] Bioferm Corp.-Stauffer Chemical Co., Thuricide WP, Lot No. D-12837.
[4] Bioferm Corp.-Stauffer Chemical Co., Thuricide 10 Dust, Lot No. D-12837.

bacillus preparations were applied as sprays and/or dusts on young cauliflower 8 to 10 inches high, with a severe infestation of cabbage loopers at Huntington Beach, California, on June 10-11, 1959. All sprays were applied at the rate of 49 to 68 gallons per acre. The plots were four 2-row beds 80 feet long. On June 12 and 16, 36 plants per treatment were examined. The results are presented in table 4.

General Chemicals 3583 was the most effective of the materials tested. However, it is of interest that, although not particularly active against larger larvae, dimethoate provided the best control of small larvae after 6 days. This may be due to the systemic activity of the compound resulting in a sufficient translocation to the site of feeding to cause an appreciable mortality of first-instar larvae.

Malathion-Perthane did not provide significantly better control than an equivalent dosage of Perthane alone. However, these compounds did give a comparatively good initial kill of large larvae, and have been commercially used in southern California for this purpose.

None of the microbial preparations was able to bring about marked reductions in the heavy looper population. The application of a wettable powder was almost totally ineffective. The bacillus dust materials also performed poorly; however, this lack of control may have been due in part to the low rates of application of the preparations, which resulted in insufficient amounts of the toxic ingredient on the plants to cause death to the feeding insects.

Test No. 5.—A test was conducted to evaluate various chemical materials and a group of newly developed bacillus preparations against the cabbage looper on recently thinned 2- to 3-inch-high head lettuce near Blythe, California, on September 23, 1959. All sprays were applied at the rate of 52 to 66 gallons per acre. The plots were four 2-row beds 100 feet long. Only one count of surviving larvae on 60 plants per treatment was made at the end of 48 hours on September 25. The results of the test appear in table 5.

The results indicate a marked improvement in the actions of some of the bacillus dust preparations which were comparable to parathion, General Chemicals 3583, and DDT-toxaphene. The Rohm & Haas and Nutrilite dusts, both of which were rated by the manufacturers at a concentration of 5×10^{9} spores per gram, appeared to be superior to the other microbial products and gave more than 80% reduction of the cabbage looper population within 48 hours.

Test No. 6.—Chemical and bacillus preparations were applied by ground power equipment to 4- to 6-inch-high head lettuce near Blythe, California, on October 14, 1959. All sprays were applied at the rate of 50 to 66 gallons per acre. The plots were four 2-row beds 100 feet long. Eighty plants per treatment were examined on October

Table 4.—Evaluation of chemical and microbial insecticides against cabbage loopers on cauliflower. Huntington Beach, California. June 1959.

Treatment	Actual Toxicant /Acre (lb.)[2]	Mean No. Loopers per 100 Plants[1] 2 Days Small	2 Days Medium	2 Days Large	6 Days Small	6 Days Medium	6 Days Large
Sprays							
Gen. Chem. 3583	1.1	5[a]	31[a]	21[abcd]	29[ab]	42[a]	12[a]
Malathion-Perthane	1.0–1.9	39[b]	112[b]	11[a]	70[bc]	115[bc]	34[ab]
Perthane	1.7	48[b]	111[b]	14[abcd]	70[bc]	126[bc]	36[ab]
Dimethoate	0.9	48[b]	154[bc]	31[abcd]	20[a]	151[bc]	25[ab]
Bacillus (7×10^{10} spg)[3]	0.85	50[b]	176[c]	39[abcd]	81[c]	188[bc]	25[ab]
Dusts							
Bacillus (5×10^{9} spg)[4]	17.1	62[b]	106[b]	11[ab]	76[bc]	140[bc]	22[ab]
Bacillus (3×10^{8} spg)[5]	22.8	67[b]	202[c]	42[bcd]	67[bc]	199[c]	42[ab]
Bacillus (2×10^{9} spg)[6]	22.8	53[b]	132[bc]	14[abc]	73[bc]	137[bc]	20[ab]
Bacillus (5×10^{9} spg)[7] -Diazinon, 4%	22.8 / 1.1	34[b]	112[b]	22[abcd]	62[bc]	115[bc]	36[ab]
Diazinon, 4%	1.1	73[b]	224[c]	39[bcd]	92[c]	157[bc]	50[ab]
Diazinon, 4% -Perthane, 5%	0.5 / 0.6	25[ab]	118[b]	39[bcd]	84[bc]	111[b]	45[ab]
Silica Gel 78	5.7	31[ab]	132[bc]	45[d]	73[bc]	115[bc]	42[ab]
Untreated		64[b]	188[c]	50[d]	84[bc]	179[bc]	48[b]

[1] Same as in table 1.
[2] Same as in table 2.
[3] Merck & Co., Inc., Agritrol WP (MB-3).
[4] Merck & Co., Inc., Agritrol 5 Dust (MB-2).
[5] Bioferm Corp.-Stauffer Chemical Co., Thuricide 10 Dust, Lot No. D-12837.
[6] Nutrilite Products, Inc., experimental microbial insecticide dust.
[7] Merck & Co., Inc., 1 lb. Agritrol WP blended with 25 lbs. of Diazinon.

Table 5.—Evaluation of chemical and microbial insecticides against cabbage loopers on lettuce. Blythe, California. September 1959.

Treatment	Actual Toxicant /Acre (lb.)[2]	Mean No. Loopers/100 Plants[1] 2 Days Small	2 Days Medium	2 Days Large
Sprays				
Parathion	1.0	3[a]	19[a]	3[abc]
Gen. Chem. 3583	1.0	14[abc]	43[bc]	14[abcd]
DDT-toxaphene	1.9–2.8	5[a]	20[a]	0[a]
Ethion	1.0	22[bcde]	75[cd]	12[abcd]
Bacillus (7×10^{10} spg)[3]	1.0	39[def]	100[cd]	12[bcd]
Dusts				
Bacillus (5×10^{9} spg)[4]	28.7	37[cdef]	94[cd]	7[abc]
Bacillus (5×10^{9} spg)[5]	28.7	14[ab]	20[a]	2[ab]
Bacillus (1×10^{9} spg)[6]	28.7	14[abcd]	51[bc]	10[abcd]
Bacillus (5×10^{9} spg)[7]	28.7	14[abcd]	24[ab]	3[abc]
Untreated		68[f]	109[d]	29[d]

[1] Same as in table 1.
[2] Same as in table 2.
[3] Merck & Co., Inc., Agritrol WP (MB-3).
[4] Merck & Co., Inc., Agritrol 5 Dust (MB-2).
[5] Rohm & Haas Co., L-69 experimental microbial insecticide blended to a dust.
[6] Bioferm Corp.-Stauffer Chemical Co., Thuricide 1-B Dust, Lot No. D-04925
[7] Nutrilite Products, Inc., Dust, Lot No. 1004.

Table 6.—Evaluation of chemical and microbial insecticides against cabbage loopers on lettuce. Blythe, California. October 1959.

Treatment	Actual Toxicant /Acre (lb.)[2]	Mean No. Loopers/100 Plants[1] 9 Days Small	9 Days Medium	9 Days Large
Sprays				
Gen. Chem. 3583	1.0	15[a]	15[abc]	6[ab]
Parathion	0.9	21[abc]	27[cde]	15[bc]
DDT-toxaphene	1.7–2.7	17[ab]	8[ab]	0[a]
Dibrom	1.0	71[fg]	56[de]	23[cd]
Bacillus (7×10^{10} spg)[3]	0.9	50[defg]	35[cde]	37[de]
Dusts				
DDT, 5% —toxaphene, 15%	2.7 / 8.2	52[defg]	14[abc]	2[a]
Bacillus (5×10^{9} spg)[4]	27	56[efg]	64[e]	33[de]
Bacillus (5×10^{9} spg)[5]	27	48[defg]	4[a]	0[a]
Bacillus (1×10^{9} spg)[6]	31	31[bcde]	23[bcd]	4[ab]
Bacillus (5×10^{9} spg)[7]	27	31[abcd]	6[ab]	0[a]
Untreated		89[g]	73[e]	52[e]

[1] Same as in table 1.
[2] Same as in table 2.
[3] Merck & Co., Inc., Agritrol WP (MB-3).
[4] Merck & Co., Inc., Agritrol 5 Dust (MB-2).
[5] Rohm & Haas Co., L-69 Dust.
[6] Bioferm Corp.-Stauffer Chemical Co., Thuricide 1-B Dust, Lot No. D-04925.
[7] Nutrilite Products, Inc., Dust, Lot No. 1004.

17 and 48 plants on October 23. The results of this test are presented in table 6.

Although the control of medium and large larvae, which were small at the time of application, was similar for both dust and spray formulations of the DDT-toxaphene mixture, the spray was definitely superior to the dust for residual control of first-instar larvae after 9 days. This may be due, in part, to the fact that sprays are more resistant to the effects of weathering than dusts.

As in the previous field trial (Test No. 5), the newer bacillus dust preparations gave effective control of the cabbage looper. The Rohm & Haas and Nutrilite dust materials again proved to be superior to the other microbial preparations and were providing effective control of the larger cabbage loopers at the end of 9 days. Some-

what less effective was the lower concentration Thuricide 1-B dust. The Agritrol dust and wettable powder materials gave poor control despite their high concentration ratings.

Test No. 7.—The final field test in the series of evaluations for the control of the cabbage looper was conducted on a planting of young head lettuce near El Centro, California, on October 16, 1959, using three chemical spray materials at the rate of 53 to 62 gallons per acre and the most promising commercial bacillus dust preparations. The lettuce plants were 4 to 6 inches high and there was a heavy looper population in the field. The plots were four 2-row beds 100 feet long. The granulated formulation of dimethoate was side-dressed by hand in a furrow which was 2 inches deep and 3 inches from the plants. The furrow was closed immediately, and the field was irrigated within the following 24 hours. On October 21 and again on October 28, 36 plants per treatment were examined. The results are presented in table 7.

Table 7.—Evaluation of chemical and microbial insecticides against cabbage loopers on lettuce. El Centro, California. October 1959.

Treatment	Actual Toxicant/Acre (lb.)[2]	Mean No. Loopers per 100 Plants[1]					
		5 Days			12 Days		
		Sm.	Med.	Lg.	Sm.	Med.	Lg.
Sprays							
Gen. Chem. 3583	1.1	11^{a}	25^{ab}	3^{a}	8^{a}	8^{ab}	3^{a}
DDT-toxaphene	2.0–3.0	14^{a}	28^{ab}	3^{a}	17^{a}	17^{bc}	6^{a}
Guthion	1.0	20^{ab}	59^{bc}	3^{a}	14^{a}	36^{c}	20^{b}
Dusts							
Bacillus (5×10^{9} spg)[3]	31	36^{ab}	98^{cd}	20^{b}	14^{a}	95^{d}	59^{c}
Bacillus (5×10^{9} spg)[4]	33	20^{ab}	20^{ab}	0^{a}	17^{a}	3^{a}	0^{a}
Bacillus (1×10^{9} spg)[5]	39	17^{ab}	36^{ab}	0^{a}	20^{a}	31^{c}	6^{a}
Bacillus (5×10^{9} spg)[6]	35	28^{ab}	3^{a}	0^{a}	6^{a}	11^{ab}	0^{a}
Granules							
Dimethoate, 3%[c]	1.0	56^{bc}	160^{d}	42^{b}	11^{a}	98^{de}	87^{c}
Untreated		95^{c}	210^{d}	39^{b}	14^{a}	185^{e}	101^{c}

[1] Same as in table 1.
[2] Same as in table 2.
[3] Merck & Co., Inc., Agritrol 5 Dust (MB-2).
[4] Rohm & Haas Co., L-69 Dust.
[5] Bioferm Corp.-Stauffer Chemical Co., Thuricide 1-B Dust, Lot No. D-04925.
[6] Nutrilite Products, Inc., Dust, Lot No. 1004.

The dimethoate granules sidedressed into the soil did not provide any pronounced control of cabbage looper larvae. However, General Chemicals 3583 continued to give control comparable to DDT-toxaphene.

The Rohm & Haas and Nutrilite bacillus dusts continued to show their effectiveness and once more gave very good control of the cabbage looper. They appeared to be holding well at the end of 12 days despite the severity of the looper population, which caused heavy damage to the lettuce plants in the untreated check plots.

DISCUSSION.—It is difficult to compare relative efficiencies of different insecticides in any except a qualitative manner in this type of test. Perhaps the largest variable affecting such a comparison is the arbitrary dosage at which each candidate insecticide is tested; although two materials may be compared at the same application rate, the expense of applying 5 pounds per acre of one may be no more than that of applying 1 pound of the other. Other variables include the age structure of the cabbage looper population being treated, the formulation tested, and the effects of climatic conditions on the initial deposit and residue of the material.

The chemical insecticides that were evaluated have been divided into three categories based on comparisons between the control provided by them and that provided by the two standards, DDT-toxaphene at approximately 2 to 3 pounds and parathion at 1 pound of actual toxicant per acre. Only one material, General Chemicals 3583 at rates from 0.5 to 1.1 pounds per acre, appeared to be comparable to the standards. The following materials at the given rates showed fair results in the tests conducted: Dibrom, Thiodan, ethion, and Guthion at approximately 1 pound; malathion-Perthane at 1.2-1.2 or 1.0–1.9 pounds; and Sevin at 1.9 pounds per acre. The remaining insecticides gave poor results: Perthane at 1.0 or 1.7 pounds, Diazinon at 1.1 pounds, Diazinon-Perthane at 0.5–0.6 pound, Silcia Gel 78 at 5.7 pounds, and dimethoate as a spray at 0.8 or 0.9 pound or as granules sidedressed in the soil at 1.0 pound per acre. When directed against first-instar larvae only, Dibrom, malathion-Perthane, Thiodan, and Sevin showed considerable promise.

Since the cabbage looper is only moderately susceptible to *Bacillus thuringiensis* var. *thuringiensis*, the microbial preparations with low levels of toxicity used in the early tests did not give satisfactory control. The results of the later tests showed a marked improvement in the effectiveness of some of the preparations as certain of the commercial firms developed products with higher levels of insecticidal activity.

The bacillus preparations from the different manufacturers vary not only in viable spore count, which is the presently accepted guide to potency, but also in insecticidal activity, which is the actual accurate indication of effectiveness against susceptible hosts.

The Agritrol wettable powder and dust products, although relatively high in spore count, were low in insecticidal activity and generally were ineffective against the looper. The early Thuricide preparations likewise gave poor control. However, the Thuricide 1-B dust possessed a higher level of toxicity and gave much better results, although it was not so effective as the later dust products of Rohm & Haas Company and Nutrilite Products, Inc., which gave very good control and displayed satisfactory residual activity. It is evident that these two microbial materials have a sufficiently high insecticidal activity to assure good results against all sizes of cabbage loopers. At the dosages used, they were as effective in quickness of kill and residual activity as the best of the chemical insecticides.

Because of the marked variation in the toxicity levels of the different materials, it is evident that the use of total spores as an indication of active ingredient per acre (Grigarick & Tanada 1959, Hall & Andres 1959) has very little meaning on a practical level. Therefore, the adoption of a theoretical level of spore count as used by McEwen & Hervey (1959) and discussed by Hall & Andres (1959) appears unsuitable. No attempt was made in this study to arrive at any conclusions concerning a standardized dosage level applicable to all of the bacillus materials. Until industry standardization is effected, it

will be necessary for each product to be considered individually with dosage levels determined and reported on the basis of the total amount of each material used per unit of crop area.

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Effects of Insecticides on the Predaceous Arthropod Fauna of Louisiana Sugarcane Fields[1]

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ABSTRACT

Several surveys showed that heptachlor applied to sugarcane fields for control of the imported fire ant (*Solenopsis saevissima* v. *richteri* Forel) caused increases in infestations of the sugarcane borer (*Diatraea saccharalis* (F.)), probably by suppressing populations of predatory arthropods. Endrin, which provides excellent control of the sugarcane borer, caused some reduction in the number of predators collected from pitfall traps. A list of the species of predators collected during the summer of 1958 from pitfall traps located at several plantations in southern Louisiana is presented. Twenty-three of the species of spiders collected had not previously been reported to occur in Louisiana. A predatory mite *Camerotrombidium* sp. was collected at four plantations. This is the first record of the occurrence of this genus in the western hemisphere.

With increasing use of the chlorinated hydrocarbon insecticides on sugarcane has come the need for evaluating their effects on beneficial arthropods, especially ground-inhabiting species. Chlordane has been recommended for control of soil insects since 1954 at the rate of 2 pounds per acre. Endrin, which was recommended in 1958 for control of the sugarcane borer (*Diatraea saccharalis* (F.)), is now widely used against this pest. As many as four applications of endrin granules at the rate of one-quarter pound of toxicant per acre are often made. In 1958 and 1959, heptachlor granules at 2 pounds of toxicant per acre were applied to several thousand acres of sugarcane land in Louisiana as part of the Federal-State campaign to eradicate the imported fire ant (*Solenopsis saevissima* v. *richteri* Forel). Although the fire ant has been almost eradicated from heptachlor-treated fields, sugarcane borer populations have been higher in those fields than in comparable untreated fields.

Little information is available regarding the importance of predatory arthropods in sugarcane borer control. Stubbs & Morgan (1902) observed the soldier beetle *Chauliognathus marginatus* (F.) feeding on borer larvae and occasionally found considerable numbers of these beetles in sugarcane fields. They also observed two species of ants attacking sugarcane borer eggs and moths but did not indicate their value as predators.

Holloway *et al.* (1928) observed earwigs preying on eggs and larvae but did not consider that they were of any importance in controlling the borer. They also reported similar observations on the Argentine ant (*Iridomyrmex humilis* Mayr.) and stated that the ants seemed to attack only eggs that had been previously parasitized by *Trichogramma minutum* Riley and only larvae and pupae that had been injured. They believed that the damage caused by ants in increasing mealybug populations and as sugarhouse and household pests more than offset any benefit from their destruction of insects. Ingram & Bynum (1941) also concluded that the value of ants as predators was more than offset by their habits of husbanding aphids and mealybugs.

In 1958 a study was initiated in southern Louisiana to determine the importance of natural enemies in suppressing borer populations and the effect of insecticides on these natural enemies. Early emphasis was placed on collecting and identifying predatory species and on surveying insecticide-treated fields to determine the density of sugarcane borer populations.

SURVEYS OF HEPTACHLOR-TREATED FIELDS.—On October 6–9, 1958, mature sugarcane stalks were examined for sugarcane borer injury in 80 fields, 40 of which had

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been treated the previous spring with heptachlor for fire ant control. Equal numbers of treated and untreated fields were surveyed in St. Mary, Iberia, and Lafayette Parishes. A sample was obtained from each field by random selection of a single stalk from each of 10 stools 3 feet apart in a single row of sugarcane. Each sample was taken about 25 paces from the nearest headland. After removal of leaves and tops, the number of internodes per stalk and the numbers tunneled by sugarcane borer larvae were recorded.

An attempt was made to select fields in each parish in which conditions other than the heptachlor treatment were similar. The same sugarcane varieties (C.P. 44/101 and C.P. 36/13) were sampled in treated and untreated fields. Ryania had been applied against the sugarcane borer in all the fields surveyed in Iberia Parish and in 36% of those in St. Mary Parish. The remaining fields had not been treated with insecticides for borer control.

Data given in table 1 show that by harvesttime 63% of the internodes of stalks from fields treated with heptachlor had been tunneled by borers as compared with 42% of those in untreated fields. The difference is significant at the 1% level of probability.

Another survey was made in early June 1959 to determine the difference between populations of first-generation borers in fields treated the previous spring with heptachlor and in those not treated. The survey included 44 fields, half of which were treated, in St. Mary and Iberia Parishes. In each field borer-killed stalks were counted in 72 row-feet (1/100 acre) in each of four rows three rows apart. Counts were made only in first- or second-year stubble cane and without regard to varieties. In each parish, fields with the same treatment histories ranged $\frac{1}{4}$ to 1 mile apart; those with different treatment histories were sometimes separated only by a road or ditch and were never more than 3 miles apart.

Data presented in table 2 show that approximately five times as many stalks were killed in the treated as in untreated fields. The difference is significant at the 1% level.

SURVEY OF ENDRIN-TREATED FIELDS.—Another survey was made in early June 1959 to obtain similar information in fields treated the previous year with endrin. Borer-killed plants were counted in 76 fields, half of which were treated, in St. Mary, Lafourche, and Assumption Parishes. Fields in which both endrin and heptachlor had been applied were not sampled.

Data in table 3 show that nearly twice as many borer-killed plants were found in untreated as in treated fields; however this difference is not significant at the 5% level.

PITFALL TRAP COLLECTIONS.—During 1958 the effectiveness of endrin and ryania for control of second- and third-generation sugarcane borer populations was compared in a randomized block experiment in which the treatments were replicated at each of seven plantations in six parishes (Long *et al.* 1959). A 2% endrin granular formulation was always applied. Forty per cent ryania granules were applied at four plantations and 40% ryania dust at the other three. Plots were approximately equal in size at each location and varied from 2 to 11 acres at different plantations. Four applications of each insecticide were made by airplane biweekly from June 23 to August 15.

Table 1.—Injury to sugarcane by the sugarcane borer in heptachlor-treated and untreated fields, October 1958.

Parish	Number of Comparisons	Average of Internodes Tunneled (%)	
		Treated[a]	Untreated
St. Mary	22	65	52
Iberia	10	61	40
Lafayette	8	62	33
Total or average	40	63	42

[a] Treated with 20 pounds of 10% heptachlor granules in the spring of 1958.

Table 2.—Number of plants killed by first-generation sugarcane borers in heptachlor-treated and untreated sugarcane fields, June 1959.

Parish	Number of Comparisons	Average Number of Borer-Killed Stalks per Acre	
		Treated[a]	Untreated
St. Mary	14	2,770	561
Iberia	8	1,512	300
Total or average	22	2,312	466

[a] Treated with 20 pounds of 10% heptachlor granules in the spring of 1958.

Table 3.—Number of plants killed by first-generation sugarcane borers in endrin-treated and untreated sugarcane fields, June 1959.

Parish	Number of Comparisons	Average Number of Borer-Killed Stalks per Acre	
		Treated[a]	Untreated
St. Mary	20	535	809
Lafourche	14	188	365
Assumption	4	131	412
Total or average	38	365	601

[a] Treated with four biweekly applications of 2% endrin granules at the rate of 12 pounds per acre. Applications were made from June to August 1958.

All plots at one plantation (St. John) were inadvertently treated with heptachlor for fire ant control prior to the first insecticide applications against the borer.

Two pitfall traps were placed about 150 feet apart in a sugarcane row near the center of each plot. Each trap consisted of a 1-quart freezer jar imbedded at ground level. Metal shields were placed over traps to exclude rain and debris. Seventy per cent ethyl alcohol plus a small amount of kerosene was used as a killing and preserving agent in the traps and was usually replenished biweekly. All arthropods trapped were removed biweekly, washed in xylene or acetone, and preserved in 95% ethyl alcohol.

The total numbers of ants collected (table 4) indicate reductions of 78% and 53%, respectively, in endrin- and ryania-treated plots. Fewer specimens were always collected in endrin-treated than in untreated plots at all plantations except St. John which had received an application of heptachlor for fire ant control. No ants were collected in plots located at this plantation. The data for St. John also indicate that Staphylinidae were much more abundant there than at other plantations. The data for Cantharidae, Carabidae, Labiduridae, and Staphylinidae

Table 4.—Number of specimens of several families of insects collected in pitfall traps in insecticide-treated plots at seven plantations in southern Louisiana, July–September 1958.

Treatment	Family				
	Cantharidae	Carabidae	Formicidae	Labiduridae	Staphylinidae
Clovelly Plantation, Lafourche Parish					
Check	5	80	12	28	8
Ryania	1	42	18	41	12
Endrin	0	126	5	24	16
Raceland Plantation, Lafourche Parish					
Check	1	1	25	12	3
Ryania	6	4	5	3	8
Endrin	1	6	3	7	4
Waterford Plantation, St. Charles Parish					
Check	1	5	9	18	5
Ryania	3	0	4	0	5
Endrin	1	1	5	0	1
Little Texas Plantation, Assumption Parish					
Check	3	1	29	0	36
Ryania	0	2	3	0	6
Endrin	4	7	7	0	7
Belleview Plantation, St. Mary Parish					
Check	0	1	50	14	11
Ryania	0	1	55	13	13
Endrin	1	5	9	11	6
St. John Plantation, St. Martin Parish[a]					
Check	2	2	0	0	133
Ryania	1	8	0	0	119
Endrin	0	2	0	0	112
Pearce Farm, Avoyelles Parish					
Check	0	5	114	0	9
Ryania	5	3	27	0	12
Endrin	1	5	23	0	13
Summary					
Check	12	95	239	72	205
Ryania	16	60	112	57	175
Endrin	8	152	52	42	159

[a] All plots were inadvertently treated with heptachlor for fire-ant control prior to insecticide applications for control of the sugarcane borer.

are too inconsistent to indicate any effect of endrin or ryania on their abundance.

A list of the arthropods collected during the period March-September 1958 at one or more of the seven plantations is presented below.

COLEOPTERA

Anthicidae
Anthicus sp.
Cantharidae
Belotus sp.
Chauliognathus marginatus (F.)
Chauliognathus sp.
Carabidae
Calosoma sp.
Dicaelus sp.
Feronia sp.
Harpalus sp.
Scarites sp.
Cincindelidae
Megacephala '(*Tetracha*) *carolina* (L.)
Megacephala (*Tetracha*) *virginica* (L.)
Coccinellidae
Scymnus (*Diomus*) *terminatus* (Say)
Colydiidae
(genus not recognized)
Elateridae
Colaulon rectangularis (Say)
Conoderus bellus (Say)
Conoderus melliculus var. *rudis* Brown
Neotrichophorus carolinensis (Schaeffer)
Lampyridae
Photuris sp.
Melyridae
Collops sp.
Staphylinidae
(Genera not recognized)

DERMAPTERA

Labiduridae
Euborellia annulipes (Lucas)
Labidura riparia (Pallas)

HYMENOPTERA

Formicidae
Iridomyrmex humilis Mayr.
Paratrechina (*Nylanderia*) sp.
Pheidole dentata Mayr.
Pheidole sp.
Ponera opaciceps Mayr.
Ponera trigona var. *opacior* Forel
Solenopsis saevissima v. *richteri* Forel
Solenopsis xyloni McCook

CHILOPODA

Lithobiidae
Neolithobius sp.

DIPLOPODA

Polydesmi
Pseudopolyclesmus serratus (Say)

ACARINA

Trombidiidae
Camerotrombidium sp.*

ARANEIDA

Agelenidae
Agelenopsis aperta (Gertsch)
Agelenopsis emertoni Chamberlin and Ivie
Cicurina arcuata Keyserling*
Coras perplexus Muma
Tegenaria pagana C. L. Koch
Wadotes hybridus (Emerton)
Anyphaenidae
Aysha decepta (Banks)
Aysha ferox Becker
Clubionidae
Castianeira descripta (Hentz)
Castianeira variata Gertsch
Chiracanthium inclusum (Hentz)*
Clubiana abboti C. L. Koch
Clubiana maritima C. L. Koch
Clubiana obesa (Hentz)*
Meriola decepta Banks*
Phrurotimpus alarius (Hentz)*
Phrurotimpus emertoni Gertsch*
Phrurotimpus minutus (Banks)*
Scotinella fratrella Gertsch*
Trachelas ruber Keyserling
Dysderidae
Dysdera crocata C. L. Koch
Epeiridae
Allepeira lemniscata (Walckenaer)

* First record in Louisiana.

Drexelia directa McCook
Larinia directa Hentz
Mangora placida Hentz
Metazygia wittfeldae (McCook)
Neoscona benjamina Walckenaer
Neoscona minima F. O. P.—Cambridge
Scoloderus tuberculifer (Cambridge)
Gnaphosidae
Herpyllus cratus Chamberlin
Litopyllus paludis Chamberlin and Gertsch*
Poecilochroa decipiens Chamberlin*
Poecilochroa famula Chamberlin*
Sergiolus meretrix Chamberlin
Zelotes (Drassyllus) gynosaphes Chamberlin*
Zelotes rusticus (C. L. Koch)
Linyphiidae
Florinda coccinea (Hentz)
Loxosceles devius Gertsch
Lycosidae
Arctosa floridiana (Banks)*
Lycosa antelucana Montgomery
Lycosa aspersa Hentz
Lycosa avida Walckenaer
Lycosa carolinesis Walckenaer
Lycosa georgicola Walckenaer
Lycosa helluo Walckenaer
Lycosa lenta Hentz
Lycosa punctulata Hentz
Lycosa rabida (Walckenaer)
Pardosa delicatula Gertsch and Wallace
Pardosa milvina (Hentz)
Pardosa montgomeryi Gertsch
Pirata suwanea Gertsch*
Pirata sylvanus Chamberlin and Ivie*
Schizocosa crassipes Walckenaer
Trochosa acompa Chamberlin
Micryphantidae
Ceraticelus bryantae Kaston*
Cornicularia vigilax Blackwell
Eperigone tridentata (Emerton)
Erigone autumnalis Emerton*
Erigone sp.
Grammonota texana (Banks)
Meioneta formica Emerton
Meioneta spp.
Mimetidae
Mimetus hesperus Chamberlin
Nesticidae
Nesticus pallidus Emerton*
Oxyopidae
Oxyopes salticus (Hentz)
Pholcidae
Pholcus phalangoides (Fuesslin)
Pisauridae
Ddomedes albineus (Hentz)
Pisaurina mira (Walckenaer)
Thanatidius dubius (Hentz)
Thanatidius tenuis Simon
Salticidae
Hentzia palmarum (Hentz)
Pelleres borealis (Banks)*
Pelleres coronatus Hentz*
Zygoballus bettini Peckham
Tetragnathidae
Leucauge venusta (Walckenaer)
Mimognatha foxi (McCook)*
Theridiidae
Lactrodectus mactans Fabricius
Paratheridula quadrimaculatus Banks*
Teutana grossa (C. L. Koch)
Teutana triangulosa Walckenaer
Theridion albidum Banks*
Thomisidae
Misumenops celer Hentz
Xysticus texanus Banks

Most of the species of Coleoptera and Hymenoptera have not been recorded previously from sugarcane fields in Louisiana. The predatory mite *Camerotrombidium* sp. is the first record of this genus in the western hemisphere. It was collected from four of the seven plantations. Twenty-three of the species of spiders listed had not previously been reported to occur in Louisiana.

DISCUSSION.—The increase in populations of the sugarcane borer following spring or early summer applications of heptachlor was apparently caused by suppression of its predators. Most of the beneficial species listed had not previously been known to inhabit Louisiana sugarcane fields. Many undoubtedly are not predators of the sugarcane borer; however the existence of an effective predator complex is indicated by the effect of heptachlor on borer populations. Wherever this insecticide has been applied against the fire ant and not followed by adequate applications of endrin, abnormally high borer populations have occurred and persisted for at least a year.

Although endrin may suppress some important predators it is much more effective against the sugarcane borer than ryania. During 1958, ryania failed to control borer infestations in heptachlor-treated fields. Those growers who did not resort to applications of endrin suffered severe losses in sugar yields.

Although ants have been considered to be detrimental because of their association with mealybugs and aphids, it is interesting to note that they were collected from both treated and untreated plots at six plantations and apparently eliminated at the plantation where heptachlor was applied for fire ant control. The species listed are all known to be general predators; some have been observed attacking the sugarcane borer.[5]

Spiders were collected in relatively high numbers at all plantations except St. John where heptachlor was applied. It is regrettable that numerical data on their abundance was not recorded.

Accurate assessment of the relative importance of specific predators will require more extensive correlation data between numbers of predators and crop damage inflicted by the sugarcane borer together with direct observations of predation.

[5] All the species of ants submitted for specific identification were determined by M. R. Smith of the Entomology Research Division, U.S.D.A., to be predaceous.

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Synergism of Carbamate Insecticides with Octachlorodipropyl Ether[1]

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Abstract

Octachlorodipropyl ether (*bis*-(2,3,3,3-tetrachloropropyl)ether), a pyrethrum synergist, was found to act as synergist of carbamate insecticides against susceptible and carbamate-selected strains of house flies (*Musca domestica* L.). Under certain circumstances synergistic effect was evident with ratios of synergist to carbamate as low as 1:20, and persisted for at least 96 hours following application of the synergist.

The synergistic effect of piperonyl butoxide and other methylenedioxyphenyl compounds on carbamate insecticides has been studied by several investigators (Moorefield 1958, Eldefrawi *et al.* 1959, 1960, Fuchs & Zschintzsch 1959, Metcalf *et al.* 1960). It has recently been demonstrated that carbamate-selected strains of house flies remain highly susceptible to the combination of carbamate and piperonyl butoxide despite their having attained high levels of resistance to the carbamate alone (Georghiou *et al.* 1961). In view of the importance of this phenomenon in the control of the house fly and other insects, further investigation of existing and potential synergists of carbamate insecticides is indicated. One such compound, 2-(3,5-dichloro-2-biphenylyloxy) triethylamine, has recently been studied (Moorefield & Tefft 1959).

In preliminary work, the present authors found that octachlorodipropyl ether (*bis*-(2,3,3,3-tetrachloropropyl) ether), a pyrethrum synergist, acts also as synergist of carbamates. The tests described here were performed in order to determine the type and extent of potentiation produced by this compound on carbamate insecticides. Octachlorodipropyl ether was described as a pyrethrum synergist by Adolphi (1958), who reported it as performing better than piperonyl butoxide in the Pete-Grady chamber but poorly in topical application.

Materials and Methods.—The methods used in rearing and treating the flies are described in detail elsewhere (Georghiou *et al.* 1961). The strains employed were the susceptible NAIDM (National Association of Insecticide and Disinfectant Manufacturers) (S-NAIDM) and a carbamate-resistant (R-MIP) (multiresistant Isolan-Pyrolan) strain produced through selection with 3-isopropylphenyl *N*-methylcarbamate. Only female flies were utilized. These were treated by the topical method on the notum with 1-microliter volumes of the test compounds in W/V acetone solution. They were subsequently held at 60° F. and mortality counts were taken 24 hours after treatment.

Octachlorodipropyl ether is referred to here as O.D.E. and 3-isopropylphenyl *N*-methylcarbamate as Compound III.

Results and Discussion.—*Toxicity of O.D.E. to House Flies.*—O.D.E. was found to be toxic to both susceptible and carbamate-resistant strains of house flies, the LD_{50}'s for S-NAIDM and R-MIP being 2.7 μg. and 4.1 μg. per fly, respectively (fig. 1,A). It was also found to possess strong knockdown properties, the KD_{50} values being 21 and 61 minutes for the S-NAIDM and R-MIP strains, respectively. KD_{50} values for Compound III alone were 8.8 and 72 minutes for S-NAIDM and R-MIP, respectively, and for the combination of these compounds 8 and 41 minutes, respectively. These tests were performed at 85 ± 2° F. The dosages used were selected to give nearly 100% mortality. In the case of R-MIP on which Compound III could not produce complete mortality, a dose of 20 μg. was employed.

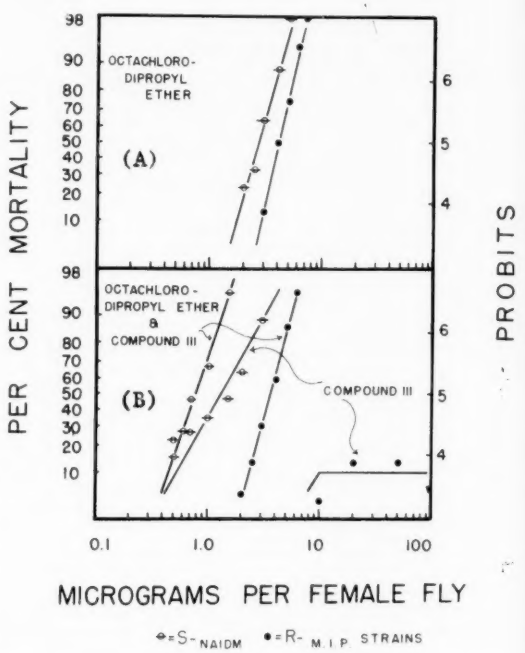

Fig. 1.—Susceptibility of S-NAIDM and R-M.I.P. (F_{13}) flies to (A) Octachlorodipropyl ether, (B) Compound III alone and in combination with octachlorodipropyl ether, in constant ratios of 1:1 and 3:1, respectively.

Activity of O.D.E. in Combination with Compound III at Various Ratios.—Dosage-mortality data for various combinations of O.D.E. and Compound III are presented in table 1. A synergistic effect of O.D.E. was evident in the case of S-NAIDM flies when a dose of 0.5 μg. of Compound III was combined with O.D.E. at a 1:1 ratio. When the dose of Compound III was increased to 1 μg.

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Table 1.—Dosage-mortality data for Compound III and O.D.E. alone and in various combinations on S-NAIDM and R-MIP house flies.

Dosage μg./Female Fly		24-Hour Per Cent Mortalities					
		S-NAIDM			R-MIP(F_{12})		
Compound III	O.D.E.	Compound III Alone	O.D.E. Alone	Both	Compound III Alone	O.D.E. Alone	Both
0.5	0.1	10		10	0		
.5	0.5		0	26			
.5	1.0		6	63			
.5	2.0		23	95		0	0
1.0	0.1	29		46	0		
1.0	0.5			79			0
1.0	1.0			90			9
1.0	2.0			99			33
2.0	0.1	61		86	0		11
2.0	0.5			98			24
2.0	1.0			100			40
2.0	2.0			100			73

Table 2.—Separate applications of Compound III and O.D.E. to different sites of the integument of house flies.

Compound, Site of Application and Dosage (μg./female fly)			
Compound III (Notum)	O.D.E. (Abdominal Sterna)	Strain	24-Hour Per Cent Mortalities
1.0	—	S-NAIDM	47
—	1.0		6
1.0	1.0		93
2.0	—	R-MIP(F_{12})	0
—	2.0		0
2.0	2.0		15
5.0	—		5
5.0	2.0		77

and 2 μg., respectively, ratios of synergist to insecticide as low as 1:10 and 1:20, respectively, could produce synergistic effect. A similar effect was also evident on the R-MIP strain.

Dosage-mortality regression lines were established for combinations of O.D.E. and Compound III at a ratio of 1:1 on the S-NAIDM strain, and 1:3 on the R-MIP strain (fig. 1,B). An approximately twofold increase in the toxicity of Compound III to S-NAIDM flies was obtained, the LD_{50} value for the insecticide alone being 1.4 μg. and for the combination 0.77 μg. Complete mortality of R-MIP flies was obtained with 10 μg. of Compound III used in combination with O.D.E., while the maximum mortality obtainable with Compound III alone did not exceed 13%.

Application of O.D.E. and Compound III to Different Sites of the Integument of House Flies.—Results of separate applications of Compound III to the thoracic nota and O.D.E. to the abdominal sterna are given in table 2. In all cases, the toxicity obtained by application of Compound III to the nota was increased when accompanied by application of O.D.E. to the abdominal sterna. It was concluded that penetration is not the only factor responsible for the potentiation of Compound III by O.D.E., and that the synergist must exercise some physiological effect which predisposes the insect to greater susceptibility to the carbamate. This point was further substantiated by a study of the effect of separate applications of the synergist at various time intervals in advance of the insecticide.

Separate Applications of O.D.E. Followed by Compound III at Various Time Intervals.—In these tests O.D.E. was applied first, followed by Compound III at various time intervals, *i.e.*, 2, 7, 24, 48, 72, and 96 hours (table 3). The synergistic effect of O.D.E. declined slowly as the time interval between the two applications was increased. This effect was evident even 96 hours after application of O.D.E., indicating a very slow recovery in the treated flies from the effect of the synergist.

Synergistic Effect of O.D.E. on Other Carbamates.—Synergistic effect of O.D.E. was demonstrated on five other carbamates in addition to Compound III. This was accomplished by treating S-NAIDM and R-MIP flies with O.D.E. on the abdominal sterna, followed by application of the insecticide to the thoracic nota. The efficiency of all insecticides tested was increased when the treatment

Table 3.—Dosage-mortality data for separate applications of O.D.E. at various time intervals in advance of Compound III on S-NAIDM and R-MIP house flies.

Compound and Dosage per Female Fly	Strain	Per Cent Mortalities 24 Hours After Last Application						
		Hours Between Application of First and Second Compounds						
		0	2	7	24	48	72	96
O.D.E. (1 μg.)	S-NAIDM	0						
Compound III (1 μg.)		13						
O.D.E. (1 μg.) then Compound III (1 μg.)			98	85	83[a]	83[b]	78[b]	78[b]
O.D.E. (2 μg.)	R-MIP(F_{12})	0						
Compound III (10 μg.)		27						
O.D.E. (2 μg.) then Compound III (10 μg.)			100	100	100	93	64	

[a] 40% mortality from Compound III alone at 24 hours.
[b] 50% mortality from Compound III alone at 48, 72 and 96 hours.

Table 4.—Synergism of various carbamates with O.D.E. (octachlorodipropyl ether) against S-NAIDM and R-MIP strains of house flies.

Compound	Dosage μg./Female Fly	24-Hour Per Cent Mortalities: O.D.E. Alone on Abdominal Sterna	Insecticide Alone on Notum	Both
S-NAIDM strain				
O.D.E.	1.0	10		
3,5-dimethoxyphenyl *N*-methylcarbamate	0.1		25	63
	0.15		43	83
3-*tert*-butylphenyl *N*-methylcarbamate	1.0		10	95
	5.0		38	100
Isolan®[a]	0.1		20	35
	0.5		30	80
Pyrolan®[b]	0.5		5	33
	1.0		25	60
Sevin®[c]	2.0		13	40
	7.0		28	53
R-MIP strain				
O.D.E.	2.0	5		
3,5-dimethoxyphenyl *N*-methylcarbamate	0.1		0	58
	0.2		0	85
	0.4		8	100
3-*tert*-butylphenyl *N*-methylcarbamate	5.0		0	30
	10.0		0	30

[a] 1-isopropyl-3-methylpyrazolyl-(5)-dimethylcarbamate.
[b] 1-phenyl-3-methylpyrazolyl-(5)-dimethylcarbamate.
[c] 1-naphthyl *N*-methylcarbamate.

was accompanied by application of O.D.E. to the abdominal sterna (table 4). When tested on the S-NAIDM strain all materials were potentiated roughly to the same extent, except for 3-*tert*-butylphenyl *N*-methylcarbamate whose potentiation was the most pronounced. Potentiation was more obvious on the R-MIP strain. This may be due in part to the fact that twice as much O.D.E. was used on this strain as on the S-NAIDM strain (2 μg. and 1 μg., respectively).

CONCLUSIONS.—Synergism of carbamates by O.D.E. is evident from the fact that the toxicity resulting from the combined application of O.D.E. and a carbamate to house flies is greater than the sum of the individual toxicities of each of the two compounds. The evidence obtained from separate applications of carbamates and O.D.E. to different sites of the body indicates that the synergistic activity of O.D.E. is not primarily due to enhanced penetration of the carbamate. Application of O.D.E. in advance of the insecticide further indicates that the effect of the synergist extends over a period of more than 96 hours. It is believed that this effect is largely the result of inhibitory action of O.D.E. on one or more enzymes responsible for the detoxication of carbamates.

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Control of European Brown Snail in Citrus Groves in Southern California with Guthion and Metaldehyde Sprays[1]

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ABSTRACT

Metaldehyde and Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl)phosphorodithioate) sprays were evaluated in field tests for their control of European brown snail (*Helix aspersa* Müller) on citrus in southern California. Metaldehyde, at higher dosages, was effective against snails infesting lemons. There was no measurable difference between snail mortality from metaldehyde wettable powder and that from aqueous suspension treatments when compared on the basis of metaldehyde equivalents. Against snails infesting Valencia oranges metaldehyde was only partially effective. The dense foliage of Valencia orange trees appeared to protect the snails falling beneath the trees from the desiccating influence of sun and wind, with the result that many snails recovered from the effects of the metaldehyde treatment. Guthion was highly effective against snails infesting both Valencia oranges and lemons. Among snails infesting Valencia oranges, mortality rates obtained with Guthion were comparable with those obtained with a standard treatment of tartar emetic and sugar. Among snails infesting lemons, mortality rates in Guthion plots were superior to those obtained in plots treated with proprietary bait composed of tricalcium arsenate, metaldehyde, and bran. Because of its demonstrated effectiveness, Guthion appears to be a promising molluscacide. Its use on citrus for other purposes or as an additive to other combined materials used in citrus spray treatments applied during periods of high snail activity would provide adequate snail control without involving prohibitive costs.

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The European brown snail, *Helix aspersa* Müller, is generally of minor importance to the production of citrus in southern California. But in individual groves and in certain coastal areas it may frequently be the most serious problem confronting the citriculturist. Losses result from snails feeding on foliage and on ripening fruit. Injury to foliage results in reduced tree vigor and retarded growth and is particularly critical in young plantings. Fruit injury results in low-graded or culled fruit. Where infestations are severe, a large part of the crop may be lost.

Control of snails on citrus crops in California was first achieved in the early 1920's with the use of poison bait composed of calcium arsenate in bran (Basinger 1923). This bait, though now modified to include metaldehyde, is still largely relied upon for snail control in citrus groves. However, the use of bait does not always give entirely satisfactory results. Many snails remain in the trees and are not attracted to the ground-distributed bait. Moreover, when bait is correctly applied following irrigations or rainy periods, its attractiveness and hence its effectiveness is shortened by rapid deterioration.

The first report of an effective spray for control of snails was made by Tubbs (1941). Tubbs and his coworkers found that a tartar emetic and sugar spray was effective against immature snails infesting trees. Persing (1942) confirmed the effectiveness of the tartar emetic and sugar spray against snails but pointed out that the cost of such a treatment would be practical only when snails occurred in epidemic numbers. In addition, he noted that the incompatibility of tartar emetic with certain of the other chemicals utilized in citrus pest control greatly limited the possibilities of integrating snail control with the control of other citrus pests.

Pappas & Carman (1955) reported that sprays containing Isolan® 5-(1-isopropyl-3-methylpyrazolyl)dimethylcarbamate) were effective against European brown snails. However, because of other considerations, Isolan was not made commercially available in this country.

Although neither tartar emetic nor Isolan has proved fully acceptable for use as a spray in snail control programs, the possibility of spraying with an entirely suitable material would offer definite advantages. Since snail activity is largely influenced by moisture, spraying may activate many snails that remain quiescent when a poison bait is applied. Furthermore, in spraying, the toxicant is applied directly to the entire snail population, but in a baiting program, the mortality rate is dependent on the number of snails that go to the bait.

It is the purpose of this paper to present results of experiments evaluating two materials for the control of the European brown snail on citrus.

MATERIALS, METHODS AND RESULTS.—Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl) phosphorodithioate), which had demonstrated molluscacidal activity in the laboratory and in a field test during 1957, and metaldehyde were the materials tested. Guthion (15%) was used as a wettable-powder formulation and as an emulsifiable concentrate containing 1.5 pounds Guthion per gallon. Metaldehyde, 25% or 50%, was used as wettable-powder formulations and as an aqueous suspension containing 50% metaldehyde.

A poisoned bait consisting of 6.75% tricalcium arsenate, 1.5% metaldehyde, and 91.75% bran or a spray containing 2 pounds tartar emetic and 4 pounds granulated sugar per 100 gallons of spray was employed as a standard treatment for comparison.

Tests were conducted in three widely separate citrus groves. Two groves were planted to lemons. Both groves were noncultivated and irrigated by sprinkler systems. The third grove was planted to Valencia oranges. It was cultivated and irrigated by gravity flow in furrows.

Spray treatments were applied manually with a conventional citrus spray unit operating at 550 pounds pump pressure. The amount of spray applied depended on tree size and variety averaging approximately 12.5 gallons per tree in the lemon groves and 16.7 gallons per tree in the Valencia oranges. The poisoned-bait pellets were hand-broadcast into the upper portion of the tree from three or four positions around the tree so the falling pellets would be dispersed evenly on the ground beneath the tree.

Posttreatment counts were obtained at specified intervals following treatment. To facilitate making these counts, the ground under all designated count trees was raked prior to treatment application to clear away empty snail shells and other debris. All snails were counted and classified as to whether they were dead, alive on the ground, or alive in the tree. All dead snails were removed from the areas of the count trees during counting periods.

Test No. 1.—This test was undertaken in a lemon grove near Carpinteria, California. Sixteen trees in a 4- ×4-tree square were used for each treatment. Four trees in the center of each plot were used as count trees to minimize the effect of snail migrations from adjacent plots or surrounding areas that were not treated. The dosages and formulations of Guthion and metaldehyde utilized in spray treatments are listed in table 1. The tricalcium arsenate-metaldehyde-bran bait pellets which served as the standard treatment were applied at the rate of 0.5 pound per tree. Mortality counts were obtained 1 and 2 weeks following treatment.

Results presented in table 1 indicate that with the exception of 1.67 pounds of Guthion and 1.0 pound of metaldehyde per 100 gallons of spray, the treatments appear to be at least as effective as the tricalcium arsenate-metaldehyde-bran bait in controlling European brown snail on lemons. Metaldehyde at 4.0 pounds and Guthion at 3.33 pounds per 100 gallons of spray were more effective than all other treatments, including the bait standard.

Test No. 2.—A lemon grove near San Fernando, California, was selected for the second test. Procedure was similar to that employed in test 1. Several plots were added by expanding the dosage range of some of the formulations of metaldehyde and Guthion that had been represented by a single dosage application in the first test. The dosage range of the bait standard (tricalcium arsenate-metaldehyde-bran) was also expanded. The bait was applied at the rates of 0.25, 0.5 and 1.0 pound per tree. The formulations and dosages of all spray treatments applied are listed in table 2. Mortality counts were made 1 and 2 weeks and 2 months following treatment and summary results of these counts are also pre-

Table 1.—Effectiveness of metaldehyde and Guthion sprays compared with standard bait applications for control of European brown snail on Eureka lemon trees at Carpinteria, California, following treatments of April 10, 1958.

Material	Amount per 100 Gallons	Number of Snails			Per Cent Mortality
		Dead	Alive (on ground)	Alive (in tree)	
		Spray Treatments			
Metaldehyde 25%, WP	1.0 lb.	2,722	823	60	75.5
	2.0 lb.	2,314	505	29	81.2
	4.0 lb.	2,238	4	31	98.5
Metaldehyde 50%, WP	1.0 lb.	1,600	214	9	87.8
Metaldehyde 50%, aqueous suspension	1.0 pt.	1,091	176	21	84.7
Guthion 15%, WP	1.67 lb.	1,282	705	59	62.7
	3.33 lb.	2,918	78	21	96.7
Guthion 1½ lb./gal., EC	3.0 pt.	1,479	234	22	85.2
		Standard Bait Treatment			
Tricalcium arsenate-metaldehyde-bran (6.75–1.5–91.75)[a]	0.5 lb./tree	1,535	21	105	92.4

[a] Per cent dry weight.

Table 2.—Effectiveness of metaldehyde and Guthion sprays compared with standard bait treatments for control of European brown snail on Eureka lemon trees at San Fernando, California, following treatments of April 14, 1958.

Material	Amount per 100 Gallons	Number of Snails			Per Cent Mortality
		Dead	Alive (on ground)	Alive (in tree)	
		Spray Treatments			
Metaldehyde 25%, WP	1.0 lb.	1,255	214	65	81.8
	2.0 lb.	1,193	66	29	92.6
	4.0 lb.	1,862	248	55	86.0
Metaldehyde 50%, WP	1.0 lb.	1,216	113	38	89.0
Metaldehyde 50%, aqueous suspension	0.5 pt.	2,275	468	125	79.3
	1.0 pt.	1,833	156	49	89.9
	2.0 pt.	1,325	52	37	93.7
Guthion 1.5 lb./gal., EC	1.5 pt.	1,147	13	74	92.9
	3.0 pt.	1,899	0	5	99.7
	6.0 pt.	1,472	0	11	99.2
Guthion 15%, WP	3.3 lb.	1,592	13	14	98.3
		Standard Bait Treatment			
Tricalcium arsenate-metaldehyde-bran (6.75–1.5–91.75)[a]	0.25 lb./tree	1,000	318	96	70.7
	0.5 lb./tree	628	76	45	83.8
	1.0 lb./tree	1,690	7	118	93.1

[a] Per cent dry weight.

sented in table 2.

It will be noted that the higher dosages of Guthion were more effective in reducing the snail population than the metaldehyde sprays or the bait treatments. The use of bait at the rate of 1.0 pound per tree was almost totally effective against snails on the ground; however, survival of tree-inhabiting snails demonstrates the critical weakness of this type of treatment. Although a limited number of snails survived the spray treatment and remained in the Guthion-treated trees, many of the snails in the trees at the time that the poisoned-bait plot was treated were not attracted to the ground and therefore were not affected by treatment. This tendency was also apparent in the results of test 1.

Test No. 3.—In this test conducted in a Valencia orange grove near Tustin, California, each plot consisted of a 3- ×4-tree block. Only the wettable-powder formulation of 25% metaldehyde and the emulsifiable concentrate containing 1.5 pounds of Guthion per gallon were used. The dosages applied are listed in table 3. A tartar emetic-granulated sugar spray was substituted for the bait as the standard treatment.

Table 3.—Effectiveness of sprays[a] with metaldehyde, Guthion, and tartar emetic-sugar for control of European brown snail on Valencia oranges at Tustin, California.

Amount per 100 Gallons	Count Periods: Weeks After Treatment	Number of Snails: Outside Tree: Dead	Number of Snails: Outside Tree: Alive	Mortality (%)	Number of Snails: Under Tree: Dead	Number of Snails: Under Tree: Alive	Number of Snails: In Tree: Alive	Mortality (%)
			Metaldehyde 25%, WP					
1.0 lb.	1	207	27	88.5	14	97	33	9.7
	2	92	18	83.6	32	93	67	16.7
	3	—	—	—	53	31	90	30.5
2.0 lb.	1	48	2	96.0	33	92	2	26.0
	2	87	11	88.8	31	82	41	20.1
	3	—	—	—	35	45	25	33.7
4.0 lb.	1	142	16	89.9	39	174	4	18.0
	2	136	4	97.1	41	110	29	22.8
	3	—	—	—	83	197	8	28.8
			Guthion 1.5 lb./gal., EC					
1.5 pt.	1	236	25	90.4	157	252	8	37.6
	2	303	50	85.8	214	99	10	66.3
	3	—	—	—	279	31	4	88.9
3.0 pt.	1	164	12	93.2	111	174	7	38.0
	2	130	12	91.5	404	99	2	80.0
	3	—	—	—	424	42	1	90.8
6.0 pt.	1	88	12	88.0	184	107	7	61.7
	2	120	6	95.2	177	72	2	70.5
	3	—	—	—	211	20	0	91.3
			Tartar emetic and granulated sugar					
2.0 lb.	1	44	0	100.0	130	1	7	94.2
4.0 lb.	2	42	1	97.7	83	5	3	91.2
	3	—	—	—	256	4	11	94.5

[a] Applied April 21, 1958.

Mortality counts were taken in two separate ground areas: (1) in a 2-foot-wide band encircling the tree just beyond the outer edge of the tree skirt, and (2) in the protected area beneath the canopy of the tree. At weekly intervals, beginning a week after treatment and continuing for 3 weeks, a different tree in each of the plots was sampled and all live and dead snails were counted. No counts were made in the ground area outside the tree the third week.

The data presented in table 3 indicate that Guthion was effective in reducing snail populations within Valencia orange trees whereas metaldehyde was relatively ineffective. When only the snails that fell to the exposed area outside the tree were considered, moderate control was obtained regardless of the material and dosages used. However, beneath the tree, treatments varied in effectiveness. In all metaldehyde plots, the percentage of mortality was low in comparison with results in the tartar emetic-sugar standard treatment. Undoubtedly, the dense foliage and lower branches of the Valencia trees prevented desiccation by sun and wind, thereby giving affected snails a chance to recover.

In Guthion plots the percentage of mortality increased as time passed. By the end of the third week following treatment there was no significant difference in percentage of snail reduction between the Guthion and the tartar emetic-sugar treatments.

Discussion.—The observed effectiveness of Guthion for the control of European brown snail infesting citrus is particularly interesting since this material is a versatile insecticide and is currently being manufactured and sold for registered use. Because it may ultimately be used for the control of other pests on citrus, Guthion sprays may also provide suitable snail control without incurring the costs of a specific treatment. In contrast to tartar emetic, Guthion is compatible with many of the materials currently used on citrus, and its inclusion specifically for snail control could be effected with a resultant saving in application cost.

Although Guthion has definite molluscacidal properties, observations on its activity against slugs would tend to indicate that the compound is not effective against these pests. In test 1, conducted near Carpinteria, the gray garden slug, *Deroceras reticulatum* (Müller) was present in all experimental plots at the time of treatment. Where metaldehyde was applied at either 4.0 pounds or at 1.0 pint per 100 gallons of spray, no live slugs were found when posttreatment counts of snails were obtained. However, live slugs were still present in all Guthion-treated plots

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An Evaluation of Insect Resistance in Broccoli, Brussels Sprouts, Cabbage, Collards, and Kale[1]

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ABSTRACT

Resistance to *Pieris rapae* (L.), *Brevicoryne brassicae* (L.), *Myzus persicae* (Sulz.), *Phyllotreta vittata* (Fab.), and *Lygus* spp. was recorded in the plant (*Brassica oleracea* L.) varieties of broccoli, Brussels sprouts, cabbage, collards, and kale. In these varieties no resistance was found to *Phyllotreta cruciferae* Goeze and *Psylliodes punctulata* Melsh. nor to *Trichoplusia ni* (Hbn.) and *Plutella maculipennis* (Curt.) which existed at low densities. No one plant variety was most or least resistant to all the insect species; each plant variety had its own pattern of resistance. Resistance of collards and kale to *P. rapae* resulted principally from increased exposure of larvae of *P. rapae* on the open leaves to the parasites, *Phryxe vulgaris* (Fall.) and *Apanteles glomeratus* (L.). Plant resistance was based on both intrinsic (physiological) and extrinsic (ecological) factors, and the interaction of both.

The focus of this investigation concerned the differences in insect abundance on *Brassica oleracea* L. varieties of cabbage, broccoli, Brussels sprouts, collards, and kale. Consideration was given also to the problem of evaluating insect resistance in plants.

Lebedev (1924) found that flea beetles of the genus *Phyllotreta* injured Brussels sprouts less than certain strains of cabbage. The relative abundance of cabbage caterpillars on cole plants was studied by Harrison & Brubaker (1943), who concluded that factors other than the plants themselves play an important role in determining the number of caterpillars on cole plants. Breakey & Carlson (1944) reported that the cabbage varieties least injured by aphids were the flathead type, whereas the most damaged were the early- and mid-season round types.

Painter (1951) lists the following three major classes of factors which are involved in the insect resistance of plants: (1) decreased preference for oviposition, food, or shelter; (2) adverse effects of the plant on the life of the insect; and (3) ability to withstand, repair, and recover from insect infestations. These factors relate to the intrinsic characteristics of the plant itself, but do not take into account the important influence that such extrinsic factors as parasites, predators, and weather exert on both the plant and animal. A plant may not possess any intrinsic resistance, but form or location of the plant may increase the exposure of its pest insect to parasites, predators, or weather, and thus indirectly determine the abundance of the pest. The interaction of the intrinsic and extrinsic factors adds to the complexity of evaluating insect resistance in plants. Harrison & Brubaker (1943) encountered difficulty in interpreting the results of their tests. In 1939, they observed 162 larvae of *Pieris rapae* (L.) on 100 Brussels sprouts plants, whereas only 42 larvae were counted on the same number of cabbage plants. But during 1941 only 35 larvae were found on Brussels sprouts, compared with 53 larvae on the cabbage plants. Why should significant differences between plant varieties in one season completely reverse in a later season? In evaluating these data Harrison & Brubaker concluded that the number of "caterpillars supported by the plants was largely due to factors other than the type of *Brassica*." More probably the trends were caused by interaction of plant and environmental factors and were not "largely due to factors other than the type of *Brassica*." The significant differences in number of *P. rapae* on the Brussels sprouts and cabbage during any one season demonstrates the importance of the plant factor.

Experimental results obtained in small plots may also be misleading when used to evaluate insect resistance. This is illustrated by Painter's (1954) work in which he found that the Pawnee wheat variety was "about half as much infested as susceptible wheats" by the hessian fly in the small experimental plots. When the Pawnee was planted in large acreages it "rarely ever attained such an infestation as occurred" in the small nursery plots. Painter lists many reasons for this decline in fly population but emphasizes the importance of weather. In addition the manner in which the wheat was planted most probably influenced these results. That is, in the nursery plots the hessian fly could maintain its population by utilizing susceptible varieties growing side by side with the resistant varieties, but when sowed in large acreages the fly had to utilize only the resistant variety and therefore could not maintain itself.

METHODS.—During the summer of 1957 in a plot 75 feet by 100 feet the following varieties of *B. oleracea* were planted: broccoli (GLF-Italian green-sprouting), Brussels sprouts (GLF-Catskill), cabbage (GLF-Danish ball-

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Table 1.—Average number of insects recorded on 20 plants per week on the different varieties of *B. oleracea* plants.

INSECTS	BROCCOLI		BRUSSELS SPROUTS		CABBAGE		COLLARDS		KALE	
	Average	±S.E.	Average	±S.E.	Average	±S.E.	Average	±S.E.	Average	±S.E.
P. rapae	1.9	0.42	2.0	0.37	2.7	0.70	0.9	0.26	1.2	0.33
P. maculipennis	0.8	0.49	0.6	0.34	0.9	0.35	0.2	0.15	0.6	0.24
T. ni	0	—	0	—	0.2	0.22	0	—	0.2	0.02
B. brassicae	56.9	30.86	89.3	52.50	48.9	31.10	40.3	19.30	61.2	30.76
M. persicae	13.0	6.90	3.0	1.70	3.6	8.66	8.8	6.26	16.0	8.76
P. vittata	29.9	15.06	41.0	15.83	49.7	23.50	61.4	30.90	58.7	34.13
P. cruciferae	3.0	1.19	7.2	2.82	8.8	2.81	7.8	2.93	7.8	2.48
P. punctulata	1.3	0.37	2.0	0.97	2.1	1.14	6.0	2.69	3.6	1.57
Lygus spp.	1.0	0.53	0.2	0.15	0.3	0.17	1.5	0.44	1.4	0.60

head), collards (Robson-Georgia), kale (GLF-Siberian). The plants were spaced 18 inches in the row, and the rows were 3 feet apart. Five rows of each variety were planted in a random manner.

The plot was fertilized with a 5–10–5 mixture applied at a rate of 2000 pounds per acre and was limed at a similar rate. The planting was maintained weed-free.

The insect species sampled and the stage counted are listed below:

Pieris rapae (L.)—(Imported cabbageworm) Fifth-instar larvae.
Trichoplusia ni (Hbn.)—(Cabbage looper) Fifth-instar larvae.
Plutella maculipennis (Curt.)—(Diamondback moth) Fourth-instar larvae.
Brevicoryne brassicae (L.)—(Cabbage aphid) Adults.
Myzus persicae (Sulz.)—(Green peach aphid) Adults.
Phyllotreta vittata (Fab.)—(Striped cabbage flea beetle) Adults.
Phyllotreta cruciferae Goeze—Adults.
Psylliodes punctulata Melsh. (Hop flea beetle) Adults.
Lygus spp.—(Plant bug) Adults.

Five weeks after planting the seeds in the plot the first insect counts were made; thereafter counts were made weekly for 8 weeks. Once each week 20 plants of each variety were selected at random and the insects present were counted. The plant surface area of the 100 plants sampled weekly was estimated by placing a piece of plastic etched in 1-inch squares against the upper surface of the leaf. This method was demonstrated to have an error of less than 10%.

The parasitism in the moths, butterflies, and aphids was studied to more completely evaluate the factors influencing insect resistance in plants. After each lepidopteron was counted, it was placed into a vial and held in the laboratory to determine rates of parasitism. All parasitized aphids were counted. When more than 100 parasitized aphids were present on a plant a minimum sample of 100 was taken; otherwise all parasitized aphids were collected to determine the parasites and their ratios.

RESULTS.—Significant differences in plant surface area among the five varieties of *Brassica oleracea* were recorded 14 weeks after planting. The order of average square inches of leaf-surface area from the largest to the smallest plant variety was as follows: collards (1180±32.1 SE), kale (845±14.9 SE), Brussels sprouts (708±60.6 SE), cabbage (645±77.2 SE), and broccoli (528±24.2 SE). The open-leaf varieties, collards and kale, significantly exceeded the other varieties in surface area. Broccoli produced the least amount of vegetation because much of its nutrition went into the immediate production of a flower head. Conformation of these varieties played an important role in plant resistance as will be shown.

The relative abundance of some insect species varied significantly with the five varieties of cole plants (table 1). Significantly (at 5% level) more *P. rapae* were present per plant on Brussels sprouts and cabbage than on either collards or kale; *P. rapae* were more abundant on broccoli than on collards. Both Brussels sprouts and cabbage produce "heads" whereas collards, kale, and broccoli are open-leaf plants. Parasitism of fifth-instar *P. rapae* larvae found on the open-leaf varieties was 100%. On the heading varieties, parasitism of *P. rapae* on Brussels sprouts was only 78% and 40% on cabbage. These results were substantiated by field observations which indicated that the parasites, *Phryxe vulgaris* (Fall.) and *Apanteles glomeratus* (L.), found and parasitized *P. rapae* on the open-leaf varieties, but had difficulty attacking larvae living in the crevices and folds of the heading varieties. In this study the low number of *P. rapae* larvae found on collards and kale was probably a result of their high rate of parasitism rather than to intrinsic resistance of the plant itself. These results emphasize the need to identify what factor or factors are responsible for insect resistance exhibited by a particular plant in the field.

The difference between the five plant varieties as to the number of both *P. maculipennis* and *T. ni* was insignificant (table 1); however, it should be pointed out that the densities of these lepidopterons was low in spite of the fact that 160 plants were sampled. The rate of parasitism of these lepidopterons did not differ with plant variety.

Using Student's t-test for paired data (Paterson 1939) in addition to the standard error comparisons, no significant difference in number of aphids on the different *B. oleracea* varieties was detected (table 1). Because of the wide fluctuations of the aphid population exact comparisons between the plant varieties were not possible. The variations in aphid populations were caused by environmental changes in temperature, moisture, and plants during the growing season and also by the erratic distribution of aphid colonies on the plants. The environmental changes caused aphid density to rise from low during midsummer to high during late summer. For example, the

Table 2.—Average infestation[a] per *B. oleracea* variety with *Brevicoryne brassicae*, *Myzus persicae*, and *Phyllotreta vittata* on a single day.

Variety	*B. brassicae* Average ±S.E.		*M. persicae* Average ±S.E.		*P. vittata* Average ±S.E.	
Broccoli	8.2	2.54	1.2	0.76	7.2	0.85
Brussels sprouts	22.0	6.71	0.8	0.33	7.8	1.20
Cabbage	13.6	10.85	0.6	0.22	11.7	0.50
Collards	7.4	4.29	2.2	0.61	13.2	1.25
Kale	11.1	8.8	4.6	0.92	15.8	0.45

[a] Number of insects per plant.

nine weekly counts of *B. brassicae* on 20 broccoli plants were 0, 0, 2, 8, 2, 22, 56, 165, and 257. To substantiate the trends, a comparison was made of the aphid populations on a single day in late summer (August 27) when aphid populations were high (table 2). When the data were thus compared significant differences were noted in aphid abundance on the five plant varieties. Brussels sprouts had significantly (at 5% level) more *B. brassicae* than either broccoli or collards. Collards and kale had significantly (at 5% level) more *M. persicae* than either Brussels sprouts or cabbage. Parasitism in each aphid species did not differ among the five plant varieties.

The difference in distribution of flea beetles among the five plant varieties was insignificant by the comparison shown in table 1. In contrast to the aphid populations, the flea beetles were most abundant during early summer and decreased in number toward late summer. To substantiate the trends in the flea beetle populations indicated in table 1, a comparison was made of beetle populations as counted on July 22 when the populations were high. The differences in number of *P. cruciferae* and *P. punctulata* were insignificant and the data were not included in table 2. Collards and kale had significantly (at 5% level) more *P. vittata* than broccoli, Brussels sprouts, and cabbage (table 2).

Lygus spp. were more abundant (at 5% level) on collards than on Brussels sprouts and cabbage (table 1); wide variation in the data prevented making any other significant comparisons.

As documented above, no one plant variety was found on which all the insect species were most or least abundant; clearly each insect species had its own pattern of abundance with reference to the five varieties listed. Such difference in pattern are caused by the specificity of each species for the five cole varieties. These differences are expected, because each species had its own requirements—environmental conditions that favor one species may cause the extinction of another. Some of the important environmental changes which occurred in this study were: day length decreased from June to August; average monthly temperatures for the period were: June 67.4°, July 67.4°, and August 63.6° F.; total monthly rainfall fluctuated from June 3.09, July 5.55, to August 1.43 inches; the plants changed in size and nutritional value; and parasite and predator populations fluctuated during the experimental period.

The results of this study should be considered with the restriction that the experiments were conducted in relatively small plots. In small plots where both resistant and susceptible plants exist side by side the insects may exist in some numbers on the resistant varieties because they can readily move to adjacent susceptible plants for food. In a large area of a pure stand of a resistant variety, the insect might not be able to maintain itself. Therefore, it is entirely possible that some of the 10% to 20% reductions in insect abundance measured in this study might become a 90% or greater reduction in the insect population when the variety is grown in a pure stand.

In summary, no one plant variety was most or least resistant to all the insect species—each had its own pattern of resistance. Plant resistance was based on both intrinsic and extrinsic factors, and the interaction of both. Clearly any evaluation of insect resistance in plants must be based on careful physiological and ecological study.

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Translocation and Stability of Chipman R-6199 in *Pinus strobus* as Related to Its Control of the Introduced Pine Sawfly, *Diprion similis*[1]

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ABSTRACT

Following trunk implantation of Chipman R-6199 (*O-O*-diethyl *S*-(2-diethylamino)ethyl phosphorothiolate (hydrogen oxalate salt)) into white pines in July 1958, translocation into needles progressed slowly throughout that current summer and by October reached highest concentrations in the needles of lower branches. Peak insecticidal concentration in needles occurred by the spring and early summer of 1959. In August 1959, the mean concentrations of insecticide in needles in the three regions of the crown were essentially equal. The observed long stability of R-6199 in white pine explains the practical control for 2 years and a third year of partial control of the introduced pine sawfly (*Diprion similis* (Htg.))

In earlier work, Coppel & Norris (1960) obtained practical degrees of control of the introduced pine sawfly, *Diprion similis* (Htg.), in eastern white pine, *Pinus strobus* L., with the systemic insecticides, Chipman R-6199 (*O,O*-diethyl *S*-(2-diethylamino) ethyl phosphorothiolate (hydrogen oxalate salt)) and Shell 3562 (3-(dimethoxyphosphinyloxy)-*N,N*-dimethylcrotonamide) as trunk implantations. To obtain more fundamental information on the nature of translocation, deposition and stability of systemics in conifers, these aspects of the insecticidal action of R-6199 in white pine were studied during 1958, 1959, and 1960. The results of this work are reported herein.

MATERIALS AND METHODS.—Four- to six-inch d.b.h. natural regeneration white pine, 20 to 30 feet tall, were utilized. The dead or decadent branches on the lower 4 to 5 feet of the trunk were pruned making the basal trunk area readily available for insecticidal implantations and leaving all trees with more uniformly vigorous crowns. Sixteen grams of Chipman R-6199 as a 50% aqueous solution were implanted into the trunk of each of three trees (one-fourth of the total dosage in each of four 0.5-inch drilled holes). Eight grams per tree were implanted on July 17, 1958, and the same dosage was added again on July 23, 1958. The holes were approximately 1.5 inches deep and slanted downward at a 45° angle to the main trunk axis. One of the four holes was centered in each of the four quarters of circumference of the basal trunk, approximately 6 to 8 inches above the soil surface. The holes were plugged with corks after application of the insecticide. Drilled and undrilled control trees were also included in the experiment.

Following treatment, one randomly chosen terminal branch, 1.5 feet long, was taken from the lower, middle and upper crown regions of each test tree at 1, 3, 7, 14, 21, 35, 79, 365, 401 and 730 days. These samples were cut into smaller pieces and each sample placed in a plastic bag with a tag bearing the appropriate data. The samples were then placed into a deep freezer and retained at −5° C. for later laboratory analysis of insecticidal content.

In analyses, the parts per million of insecticide in 1957, 1958, 1959 and 1960 needles were determined using the cholinesterase-inhibition technique of Casida *et al.* (1956) with certain modifications. Using this technique, 5 grams of plant tissue (needles) were homogenized in 100 ml. of distilled H_2O in a Waring Blendor for 5 minutes. The homogenate was then expressed through four thicknesses of cheesecloth, adjusted to pH 8 with 0.1 N NaOH and various volume aliquots of this extract were then utilized in the analyses. The aliquots were added to 1.5 ml. of whole human blood (adjusted to pH 8) and the total volume increased to 5 ml. with distilled H_2O. Tubes containing the test solutions were then incubated for 1 hour at 37.5° C. in a controlled temperature water bath. At the end of this period, 1 ml. of each test solution was transferred to a 5-ml. beaker containing 1 ml. of pH 8 buffer solution (sodium barbital, 0.036M; potassium chloride, 1.20M; and potassium dihydrogen phosphate or potassium phosphate monobasic, 0.008N). The combined 1 ml. of test solution and 1 ml. of buffer were then incubated for 10 minutes at 37.5° C. in the temperature bath. After this, the test mixtures were returned to room temperature and the pH of each was determined using a Beckman Model G pH meter. To each beaker, 0.20 ml. of 0.66 M acetylcholine chloride was added and the contents shaken carefully and returned to the temperature bath for 2 hours. The final pH of each test solution was then taken and the percentage of enzyme inhibition was calculated using the formula:

$$\% \text{ Inh.} = \frac{\Delta \text{ pH Control} - \Delta \text{ pH treated}}{\Delta \text{ pH Control} \times 100}$$

To relate parts per million of the insecticide in the plant tissue to percentage of cholinesterase inhibition in the 30% whole human blood, a standard curve of percentages of inhibition was established from tissue extracts where various known parts per million of insecticide were added to 5 grams of plant tissue and homogenized. This curve, graphed on semi-log scale, is presented in figure 1. Each point on the curve is the mean of eight replicates.

All test samples were run at dilutions yielding percentage inhibitions between 10 and 60; the straight-line portion of the curve. There were 12 such determinations per crown position per sample date.

RESULTS.—Within 24 hours after implanting 8 grams of R-6199 into the trees on July 16, 1958, up to 15.0 p.p.m. of insecticide were present in the 1957 needles. The mean concentrations in the various portions of the tree were not significantly different (fig. 2). Though an additional 8 grams of insecticide per tree were implanted

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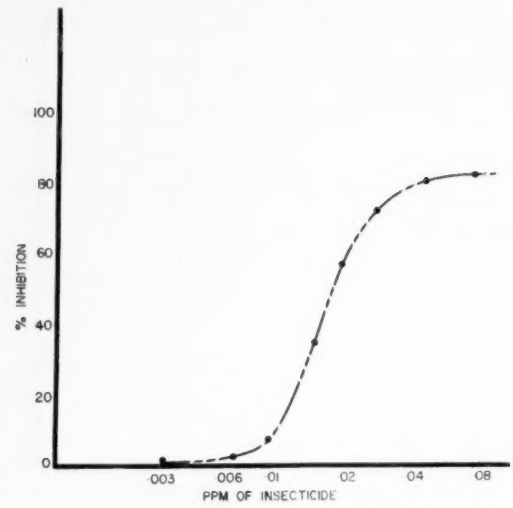

FIG. 1.—Percentages of cholinesterase inhibition in 30% whole human blood samples containing various known parts per million of Chipman R-6199 in homogenized pine needles, analyzed according to Casida *et al.* (1956) with modifications.

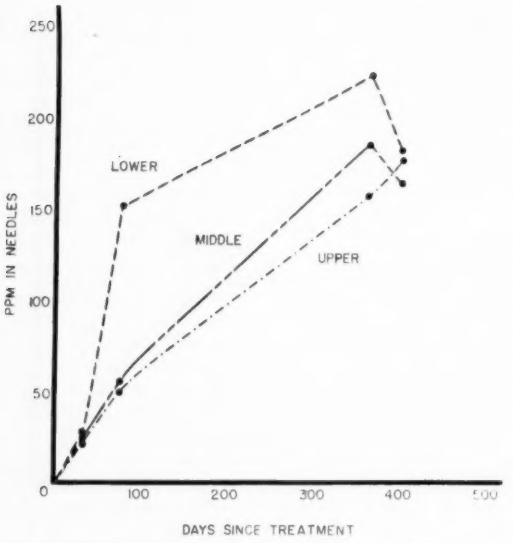

FIG. 2.—Mean parts per million of Chipman R-6199 in 1957 and 1958 needles of lower, middle and upper crown of white pines at various time intervals after treatment.

in the old holes 7 days after the initial dosage, the concentration of R-6199 in 1957 needles did not exceed 20.5 p.p.m. by 14 days after the initial treatment.

On August 21, 5 weeks after treatment, the needles sampled contained as much as 34.0 p.p.m. At this date, comparative determinations on new 1958 and old 1957 needles showed no consistent differences between the concentrations of the insecticide in these needles. By this time the 1958 needles were fully expanded. In the final samples during 1958 taken on October 3 (79 days after treatment), up to 254.0 p.p.m. of insecticide were found in needles in the lower portion of the crown. As indicated by the large increase in mean concentration of the insecticide in lower branches, larger amounts of the insecticide were moving upward in the tree at a slow rate, being deposited in the needles, but in widely varying concentrations. The concentrations of insecticide in the middle and upper crown positions showed means of approximately one-third the concentration in the lower branches. One explanation that may be suggested for this slow movement of the insecticide upward in the tree is that the major period of spring growth in the white pine, when upward sap and nutrient movement occurs at a relatively rapid rate, had passed when the implantations were made.

The uniformly large increase in mean insecticide concentrations in the various crown positions in samples of July 16, 1959, would seem to support the importance of the spring growth period as a time of much more rapid upward movement in pine trees. Another aspect in the observed rates of insecticide uptake in these trees that needs consideration is the probable effect of the accumulation of the insecticide around the 1.5-inch deep implantation holes. These holes in 6-inch d.b.h. trees penetrate into limited or nonfunctional woody tissues as far as translocation is concerned. As these holes were slanted downward at 45° to create a reservoir, the insecticide would, of course, move downward into the hole as movement into the wood occurred. No wood cores from the area surrounding the implantation sites were taken, but analysis of such tissues would have clarified the magnitude of any reservoir effect in essentially nonfunctional wood.

During the period between July 16 and August 21, 1959, the mean concentration of insecticide in 1958 needles in the lower and middle crown decreased, but increased in the upper crown. This situation may have been caused by the continued upward movement of the insecticide in the tree to those areas of greatest transpirational, photosynthetic and general physiological activity. During the early portion of this period, final expansion of 1959 needles occurred and comparative analyses of 1958 and 1959 needles taken on August 21, 1959, showed only a mean 32.8% as much insecticide in the new (1959) needles as in the older (1958) ones. Assays of 1960 needles at 730 days showed a further reduction in insecticide concentrations to less than 1 p.p.m.

The data on concentration (1957 and 1958 needles) presented in figure 2 indicate that the concentration of R-6199 or its equivalents reached a peak in needles of the tree crown by 1 year after implantation. Beyond this point, needle drop, tree growth and insecticide breakdown caused a continued decrease in insecticide concentration in each set of needles.

The observed range in insecticide concentration in various branches within the same crown region of a tree clearly explains the variation in mortality of introduced pine sawfly larvae as reported earlier by the authors (Coppel & Norris 1960). The residual concentrations of

the insecticide or its equivalents at 401 and 730 days after implantation clearly explain the practical control of the sawfly through two summers and the partial control during the third year.

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Control of Pea Enation Mosaic in Peas with Insecticides[1]

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ABSTRACT

In 1955 foliage applications of parathion and demeton were tested on Perfection and Pluperfect varieties of peas in four plantings at two locations. Three applications of parathion applied at weekly intervals reduced the incidence of pea enation mosaic (PEM) in most instances. Other treatments (two applications of parathion 10 days apart; one application of parathion followed 10 days later with demeton; two applications of demeton) gave more variable results depending upon the timing of the first application. The nonpersistent types of virus diseases (stunt and streak) were controlled, when they were prevalent in the same manner as PEM. Increased yields were obtained in the treated plots but not always of the magnitude expected from the degree of virus disease control obtained. In 1956, two tests were conducted on Pluperfect peas using foliage applications of parathion and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate) in combination with seed treatments with demeton. Three applications of Diazinon or parathion were the most effective although the results were not as spectacular as in 1955. This ineffectiveness is attributed to poor timing of the first application. Demeton seed treatment appeared to reduce pea stands. Yield increases were usually obtained with the insecticidal treatment but these increases often were not as sizable as the degree of virus control obtained would indicate.

Within recent years, the pea aphid (*Macrosiphum pisi* (Harr.)) is seldom so abundant in pea fields of western New York that insecticidal control is necessary to prevent direct feeding injury. Indirectly, however, it is injurious to peas in its role as a vector of virus diseases, the most prevalent and probably the most important of which is pea enation mosaic (PEM) (Schroeder 1951, McEwen & Schroeder 1956). Huckett (1945) showed that the incidence of virosis in pea was reduced following the use of rotenone for pea aphid control although the specific virosis was not indicated. More recently, Swenson *et al.* (1954) demonstrated control of PEM with organic phosphate sprays and briefly discussed the economics of such control in peas.

The literature pertaining to the control of plant virus diseases through control of their insect vectors has been reviewed recently by Broadbent (1957). From this review, it may be concluded that in many instances the incidence of virus diseases where the virus-vector relationship is of the "persistent" type (Watson & Roberts 1939) can be substantially reduced through the control of the insect vectors. This is true especially where spread is primarily within a given crop planting. Vector control has been much less effective in controlling virus diseases of the "nonpersistent" type (Watson & Roberts 1939).

Several species of aphids are known to transmit PEM (Osborn 1935, Chaudhuri 1950). Of these, the pea aphid is the only one regularly found in western New York pea fields and is believed primarily responsible for virus spread in this crop. The fact that the incidence of PEM varies greatly from year to year and from district to district and is not closely correlated with the abundance of pea aphids (McEwen & Schroeder 1956) indicates that proximity to suitable overwintering virus reservoirs is of great importance. The virus is not seed borne in pea and, hence, primary inoculum in this crop must be brought in by alatae migrating from virus-infected overwintering hosts. Since a viruliferous pea aphid can infect a pea plant within a 15-second feeding period (McEwen *et al.* 1957), this primary infection by migrating alatae cannot be prevented by the application of existing insecticides to the crop. Thus, the successful control of pea enation mosaic, at least theoretically, depends to a large degree on preventing the build-up of apterae in the pea field. Such prevention should decrease spread of the virus from those plants inoculated by the migrating alatae. In addition, it may be possible to kill the alatae before they have moved from the original plant attacked and, thus, localize and reduce the number of plants infected by these spring migrants.

METHODS.—In 1955, Pluperfect and Perfection varieties of peas were planted on April 18, 29, May 9, and 23 at two locations. The two varieties were planted in plots, 24×55 feet in a split-block design. Four foot fallow alleyways were left between plots and 15-foot roadways between replicates. In planting I (April 18), 10 pea plants infected with pea enation mosaic in the greenhouse were planted in the alleyway between plots to provide a source of virus inoculum. In each replicate, five untreated plots

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were used as buffers between the treated plots. The four spray treatments, arranged as a 4×4 Latin square, included: 1) two applications of parathion with a 10-day interval, 2) three applications of parathion with 7-day intervals, 3) two applications of demeton with a 14-day interval, 4) one application of parathion followed 10 to 14 days later by an application of demeton. Parathion (25%) emulsifiable concentrate was applied at the rate of 1 pint (¼ pound) per acre per application. Demeton (26.1%) emulsifiable concentrate was applied at the rate of 1 pint (¼ pound) per acre per application in the two treatments of demeton and 1 quart (½ pound) per acre per application in the combination parathion-demeton treatment. Sprays were applied with a tractor-mounted, low-gallonage sprayer equipped with a 12-foot brush-type boom which delivered about 20 gallons of spray per acre. Tractor wheel damage was equalized in all plots. All plots in each planting received one application of parathion for pea weevil control on June 13, 20, July 1, and 5 for plantings I–IV, respectively. The variation in application dates was required because of variation between blocks and varieties in maturity or blossoming.

Virus incidence data were based on four samples (1 sq. yd. per sample) randomly selected from each plot 2 days previous to harvest. Disease plants were classified purely on expressed symptoms into four categories: enation, stunt, streak and miscellaneous. In the case of the stunt and streak categories, several virus diseases were probably involved and no attempt was made to separate them other than by symptoms. Diseased plants whose symptoms were indefinite or unknown were placed in miscellaneous category. This category consisted mainly of plants showing incipient pea enation mosaic. A small number of plants showed definite symptoms of two virus diseases and were classified in both categories. Harvest stand counts were obtained from the samples taken for virus indexing.

Each plot was harvested by variety and vined by the usual commercial practice. The Pluperfect variety was harvested in the several plots on June 24 and 27, June 30, July 5, and July 14. Similarly, the Perfection variety was harvested on June 27 and 30, July 2 and 4, July 8, and July 18. Pea maturity was determined from tenderometer readings on subsamples of washed shelled peas. Yields were adjusted to a common tenderometer value 90, 69% of maximum yield for the Perfection variety (Sayre 1952). The pods were removed from the plants sampled for virus incidence, classified as to diseased or full pods, counted and weighed.

In 1956, Pluperfect peas were planted on April 25 and May 21 at the same location. Plots 24×35 feet had 4-foot alleyways between them and 12-foot roadways between replicates. Paired treated plots were buffered on either side by untreated plots (4 per replicate). The six insecticidal treatments, arranged as a 6×6 Latin square, were: 1) three applications of parathion applied at 7-day intervals, 2) two applications of Diazinon[8] (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) applied at a 10-day interval, 3) three applications of Diazinon applied at 7-day intervals, 4) demeton preplanting seed treatment, 5) demeton preplanting seed treatment plus one application of parathion to the foliage, 6) demeton preplanting seed treatment plus one application of Diazinon to the foliage. Sprays were applied to the foliage at the same rates and by the same method as described for 1955. Demeton seed treatments were applied as a slurry to the seed at the rate of 1 ounce (actual toxicant) in ½ pint of 2% methocel solution (Howe *et al.* 1952) per bushel of seed (54 pounds). Diazinon (25%) emulsifiable concentrate was used at the rate of 1 pint (¼ pound) per acre per application. Emergence counts, virus incidence data, and harvest stand counts were based on two samples (1 sq. yd. per sample) randomly selected from each plot. Diseased plants were classified as in 1955. All plants in planting I were harvested on July 6. In planting II, the untreated plots were harvested on July 18 and the treated plots on July 20 (variation in maturity). Yields were adjusted to a common tenderometer of 105, 83% of maximum yield.

RESULTS AND DISCUSSION.—In spite of a very low pea aphid population in 1955, pea enation mosaic (PEM) —as measured by its occurrence in the untreated buffer plots—occurred in 20% to 54% of the plants (table 1). Apparently a low aphid population does not necessarily ensure a correspondingly low incidence of enation mosaic in pea. This situation is frequently encountered in the pea-growing areas of New York. PEM constituted 43% to 68% of all plants infected with viroses and for some unknown reason was more prevalent in plantings III and IV of the Perfection Variety. In most instances, stunt and streak types of virus disease were of minor importance. The per cent of plants in the miscellaneous category was fairly stable and appeared to be independent of planting date, variety or total virus disease incidence.

Pea viroses were controlled, to varying degrees, by each of the four insecticidal programs tested in 1955 (table 1). Three applications of parathion applied at weekly intervals was the outstanding treatment tested, giving large reductions in the incidence of PEM in every instance under the varying conditions of 2 varieties on 4 different planting dates at 2 locations. The level of PEM incidence in these parathion-treated plots remained fairly constant in spite of the large variations in the untreated buffer plots and suggests that this treatment was highly effective in preventing secondary virus inoculations. This seemed true even though the application timing varied between the four plantings. In plantings I and II of the Pluperfect variety, two applications of parathion gave almost as good control of PEM spread as did three applications but was not as effective in the other instances. Where only two applications of parathion were used the timing of the first application was more critical than where three applications were employed. This was true particularly with the Perfection variety since timing of the first application was based primarily on growth conditions in the slower growing Pluperfect variety. Nevertheless, this did not prove to be particularly detrimental. The less critical demands in timing from the three applications of parathion program make it more practical from the commercial standpoint. Two applications of demeton seemed to have some of the same faults as two applications of parathion—primarily the necessity for more critical timing. However, in plantings II and IV of the Perfection variety two appli-

Table 1.—Results of test to control the spread of pea virus diseases through control of their vector, the pea aphid. Geneva, N. Y., 1955.

PLANTING	TREATMENT	TREATMENT DATE	PER CENT PLANTS INFECTED					AVERAGE	
			Enation	Stunt	Streak	Miscellaneous	Total	Yield of Shelled Peas (lb./a.)	Plant Stand (harvest) per 2 Sq. Yds.
			Pluperfect variety						
April 18 (I)	Untreated		51.1	1.8	2.5	19.6	73.5	1,566	177
	2 parathion	5/20, 5/31	13.2	0.6	0.0	7.4	20.9	1,478	167
	3 parathion	5/20, 5/27, 6/6	11.0	1.0	0.3	9.0	20.7	1,689	166
	2 demeton	5/20, 6/3	15.5	1.1	0.6	11.7	28.2	2,528	179
	Parathion +demeton	5/20 6/3	20.0	0.7	0.5	8.0	28.7	1,110	178
L.S.D.[a]	19:1		5.1	n.s.	n.s.	n.s.	n.s.	n.s.	2.2
April 29 (II)	Untreated		45.2	4.3	3.1	20.0	69.0	2,449	180
	2 parathion	5/23, 6/3	10.9	0.6	2.1	10.2	20.5	2,718	181
	3 parathion	5/23, 5/31, 6/7	8.8	1.8	0.6	8.7	21.5	2,755	180
	2 demeton	5/23, 6/6	7.6	1.6	0.7	7.7	17.5	2,627	178
	Parathion +demeton	5/23 6/3	11.2	2.0	0.6	8.9	22.5	3,110	159
L.S.D.[a]	19:1		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	17
May 9 (III)	Untreated		21.4	5.9	2.3	19.2	47.7	2,363	171
	2 parathion	6/1, 6/13	10.6	2.8	0.8	8.6	23.4	2,582	149
	3 parathion	6/1, 6/8, 6/15	5.3	1.7	0.5	9.8	18.1	2,719	179
	2 demeton	6/1, 6/15	8.7	1.4	0.3	10.5	20.5	2,406	162
	Parathion +demeton	6/1 6/13	8.9	2.5	0.6	9.2	20.2	2,433	191
L.S.D.[a]	19:1		2.9	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
May 23 (IV)	Untreated		31.4	4.8	4.9	21.8	62.0	1,284	164
	2 parathion	6/3, 6/13	19.1	3.1	1.9	10.2	33.6	1,315	167
	3 parathion	6/3, 6/10, 6/17	10.8	2.8	1.8	9.0	23.4	1,680	166
	2 demeton	6/3, 6/17	15.2	4.0	3.6	9.6	31.4	1,451	158
	Parathion +demeton	6/3 6/13	13.6	3.5	2.0	13.7	31.7	1,662	176
L.S.D.[a]	19:1		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
			Perfection variety						
April 18 (I)	Untreated		53.9	9.3	1.6	22.7	86.2	965	150
	2 parathion	5/20, 5/31	17.0	3.2	0.5	19.4	39.4	1,320	142
	3 parathion	5/20, 5/27, 6/6	9.4	2.0	0.0	20.6	30.0	1,794	148
	2 demeton	5/20, 6/3	18.2	8.7	2.5	24.9	55.7	1,547	129
	Parathion +demeton	5/20 6/3	14.9	2.1	0.9	14.6	30.0	1,240	147
L.S.D.[a]	19:1		n.s.	4.6	1.1	n.s.	17.1	n.s.	n.s.
April 29 (II)	Untreated		48.6	2.7	8.6	25.1	82.6	1,601	141
	2 parathion	5/23, 6/3	13.8	0.7	1.8	10.3	24.3	2,424	138
	3 parathion	5/23, 5/31, 6/7	9.7	1.4	2.4	14.9	27.8	2,171	137
	2 demeton	5/23, 6/6	8.0	1.4	1.8	15.7	26.2	2,347	146
	Parathion +demeton	5/23 6/3	10.6	0.4	2.1	12.5	25.3	2,239	128
L.S.D.[a]	19:1		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	9.8
May 9 (III)	Untreated		33.3	12.0	5.7	24.4	69.8	1,586	128
	2 parathion	6/1, 6/13	15.2	4.6	2.8	13.8	36.9	1,703	115
	3 parathion	6/1, 6/8, 6/15	8.9	2.7	4.3	13.2	24.3	2,000	124
	2 demeton	6/1, 6/15	14.3	5.0	3.3	18.6	35.7	1,872	134
	Parathion +demeton	6/1 6/13	17.8	5.1	2.8	13.4	37.2	1,782	136
L.S.D.[a]	19:1		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
May 23 (IV)	Untreated		49.9	5.6	7.0	20.3	79.0	952	145
	2 parathion	6/3, 6/13	22.6	5.8	3.9	8.7	37.9	1,151	142
	3 parathion	6/3, 6/10, 6/17	9.9	2.7	2.2	7.8	22.4	1,359	141
	2 demeton	6/3, 6/17	11.0	2.0	1.5	11.3	25.5	1,356	135
	Parathion +demeton	6/3 6/13	8.4	2.4	2.8	9.4	22.8	1,313	168
L.S.D.[a]	19:1		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[a] Between treatment means, data from untreated plots not included in analysis.

cations of demeton gave reduction in PEM equal to that obtained with three applications of parathion. Apparently, the translocation of demeton to the rapidly growing terminals, where the pea aphid prefers to feed, is either not sufficiently rapid or, more likely, not in effective quantities (Tietz 1954). The combination treatment of parathion and demeton gave erratic control of PEM, varying from poor (planting I of Pluperfect and planting III of Perfection) to excellent (planting IV of Perfection). Theoretically, this treatment should have been very effective. Poor results may be explicable on the basis of incorrect timing or improper interval between applications.

The stunt and streak types of virus disease responded to treatment where the prevalence of this disease was large enough to evaluate. Three applications of parathion gave outstanding control of stunt in all cases and was particularly effective in plantings I and III of the Perfection variety. The three other treatments gave some control of stunt but were less effective than the three applications of parathion treatment. Streak was also controlled by insecticidal treatment. The heaviest occurrences were in plantings II, III, and IV of the Perfection variety. All of the four treatments appeared to be equal in controlling this type of virus disease. Since stunt and streak types belong to the "nonpersistent" category of virus diseases (aphid retains its infectivity for only a short duration of time), it was somewhat surprising that these insecticidal programs, with long intervals between applications and low aphid populations present, gave any control at all.

The miscellaneous category included about one-third of the diseased plants observed. Most of these plants showed rather mild symptoms probably incipient PEM, although viruses of the bean yellow mosaic group show similar symptoms on some varieties. Such infections probably occurred after the insecticidal applications for weevil control were terminated. Under these circumstances, it would be expected that previous insecticidal treatment would have only a minor influence on prevalence but this was true only in the case of planting I of the Perfection variety. All treatments seemed to be effective in controlling the viroses classified as miscellaneous although the response was not as spectacular as for PEM nor did it reflect entirely a response parallel to that obtained with PEM. The degree of control obtained did not appear dependent on the treatments' effectiveness in controlling the spread of viroses in the other categories. This is probably because of other compensating factors, such as all receiving the last application about the same time and the use of identical treatments for weevil control.

The lack of significance in control of virus spread between insecticidal treatments was not too disturbing as all treatments were planned as practical control programs, based on previous investigation (Swenson *et al.* 1954). The better response obtained in some plantings with certain treatments is believed to be caused by differences in timing. Where the first application was applied shortly after emergence (plantings III and IV), three applications of parathion was the only treatment consistently effective. The apparent difference in virus incidence between Pluperfect and Perfection varieties could have been caused by a vector preference but the thinner stand of plants in the Perfection variety might have been responsible. In actuality, this difference is illusory since the number of infected plants per square yard is approximately the same for both varieties (table 2). The difference in stand density creates this effect only when the data are treated on a percentage basis.

In spite of an exceedingly dry, hot and otherwise unfavorable growing season for peas, the yield of shelled peas was satisfactory (table 1), but varied considerably between planting dates and between varieties. The variation in yield between planting dates is expected and is caused by seasonal environal conditions. In most instances, yields were increased in plots receiving insecticidal treatment but these increases were not necessarily directly correlated with the degree of virus control obtained. The magnitude of yield increase was often large percentage-wise but not poundwise. The results from the untreated and treated plots were not directly comparable since the untreated was the average of 20 plots and in the case of the treated plots there were only four. In addition, the untreated plots were not randomly distributed in the experiment but were placed as guard or buffer plots. The most effective treatment, three applications of parathion, outyielded all other treatments in 6 of the 8 comparisons (Pluperfect planting I and Perfection planting II, the two exceptions). Since these data (table 1) do not give a direct comparison between the untreated and treated plots, a total correlation was run (table 3) by Fisher's method. In spite of the variations in harvest stand, it did not appear to be a factor in yield.

Table 2.—Plant stand counts in untreated plots of peas. Geneva, N. Y. 1955.

Planting Date	Average Stand Pea Plants/2 Sq. Yds.[a]					
	Perfection			Pluperfect		
	All Treatments	Infected with Virus		All Treatments	Infected with Virus	
		Number	Per Cent		Number	Per Cent
I—April 18	150	129	86	177	130	74
II—April 29	141	116	83	180	124	69
III—May 9	128	89	70	171	81	48
IV—May 23	145	114	79	164	102	62
Average	141	112	80	173	109	63

[a] Rounded to nearest whole number.

Table 3.—Coefficients of correlation between stand of pea plants, total number of plants infected with virus, number of plants infected with pea enation mosaic, and yield of shelled peas. Geneva, N. Y. 1955.

	Plantings				
	I	II	III	IV	Average
Pluperfect variety					
Stand×yield	.0566	.1850	−.1213	.1545	.0693
Total virus×yield	.0953	−.2489	−.2723	−.5592	−.2620
Total virus×stand	.3540	.2462	.1986	.2146	.2545
Enation×yield	−.1572	−.2155	−.1029	−.6523	−.3037
Perfection variety					
Stand×yield	.1186	.0085	−.0539	.0795	.0395
Total virus×yield	−.2643	−.3072	−.5767	−.7172	−.4889
Total virus×stand	.2813	.3273	.4935	.0209	.3006
Enation×yield	−.3288	−.3043	−.5307	−.7479	−.5136

Fisher's Method (1930).
N = 34 P = .01 r = .4182.

Table 4.—Results of test to control the spread of pea virus diseases through control of their vector, the pea aphid. Geneva, N. Y., 1956.

PLANTING	TREATMENT	DATES OF TREATMENTS	PER CENT PLANTS INFECTED					YIELD OF SHELLED PEAS (LB./A.)	PLANT EMERGENCE PER SQ. YD.	HARVEST PLANT STAND PER SQ. YD.
			Enation	Stunt	Streak	Miscellaneous	Total			
April 25 (I)	Untreated		6.7	1.1	2.7	7.1	17.5	3,419	85.1	85.1
	3 parathion	5/22, 6/4, 6/14	8.1	1.4	2.2	4.0	13.0	3,733	81.4	90.1
	2 diazinon	5/22, 6/8	6.5	0.7	2.5	5.5	14.7	3,800	85.2	92.7
	3 diazinon	5/22, 6/4, 6/14	4.0	0.7	1.2	4.2	10.9	3,858	84.0	86.4
	Demeton		7.3	1.1	3.2	4.8	19.1	3,574	79.7	81.4
	Demeton +parathion	6/14	6.6	1.1	2.0	5.5	12.0	3,223	81.6	83.1
	Demeton +Diazinon	6/14	8.6	1.9	2.8	5.5	18.8	3,437	79.6	78.8
L.S.D.[a]	19:1		n.s.			n.s.	6.3	n.s.	n.s.	6.5
May 21 (II)	Untreated		32.0	3.0	2.8	8.2	46.2	3,401	86.0	86.8
	3 parathion	6/14, 6/21, 6/28	15.1	2.0	0.9	4.6	22.7	3,703	85.8	85.2
	2 diazinon	6/14, 6/28	22.0	1.6	1.5	4.7	29.7	3,518	91.1	93.7
	3 diazinon	6/14, 6/21, 6/28	14.9	2.9	1.3	5.8	25.6	3,999	83.3	85.1
	Demeton		22.1	1.9	1.7	7.6	33.3	2,863	77.3	77.8
	Demeton +parathion	6/28	19.8	2.1	1.2	7.8	31.2	3,424	76.6	76.7
	Demeton +Diazinon	6/28	23.8	2.8	2.3	8.6	37.5	3,575	74.5	77.2
L.S.D.[a]	19:1		6.5	n.s.	n.s.	n.s.	n.s.	430	7.9	7.7

[a] Between treatment means, data from untreated plots not included in analysis.

The yield of full pods obtained from the plants taken from the virus incidence samples was directly proportional or correlated to the yield of shelled peas. The yield of diseased pods was correlated with virus incidence and yield of shelled peas. Since these data were so voluminous and did not add either to the virus incidence or yield data they have not been included.

Stand was not significantly correlated with virus incidence in the Pluperfect variety but it was so correlated in one instance in the Perfection variety (planting III). In three instances, yield was negatively correlated with virus incidence and PEM seemed to be the virus disease responsible.

In 1956, the prevalence of virus diseases was not as extensive as during 1955, particularly in the April 25 planting (table 4). As in 1955 the aphid population was so low that population counts were not used as a criterion of treatment efficiency. PEM was the most prevalent virus disease in the plots with stunt and streak being of minor importance. Three applications of either Diazinon or parathion were the most effective treatments tested although the control obtained was not as spectacular as that obtained during 1955 with parathion. It is difficult to determine the exact cause of this poorer response to treatment, particularly in the April 25 planting. It would be convenient to assess the ineffectiveness to improper timing (late application) but this may be too simplified an explanation. In any event, it does indicate that the prevention of virus spread in peas through spraying is of a more critical nature than had heretofore been supposed. The seed treatment with demeton was not as effective as foliar applications even when followed by a late application of either parathion or Diazinon. Demeton seed treatment depressed the emergence especially in the May 21 planting. The lower stand count at emergence as contrasted to that at harvest is caused by delayed emergence as well as sampling deviations or errors. Yields do not necessarily follow the variance in stand but were highest where the virus control was greatest.

It was possible to control the spread of pea virus diseases through the control of their vector, the pea aphid. The greatest response to insecticidal treatment was obtained with PEM although the nonpersistent types (streak and stunt) responded when they were prevalent. Timing so that the first application was made soon after plant emergence was important in reducing the number of primary infections. The increase in yield of peas caused by a reduction in the occurrence of PEM was not always sufficiently large to justify the cost of spray applications. However, the most effective treatments appear to be economically sound even when the virus incidence is at a reasonably low level.

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A Laboratory Evaluation of Ethion with Other Acaricides Against the Adult Two-Spotted Spider Mite, *Tetranychus telarius*[1]

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ABSTRACT

Several acaricides were evaluated against the adult two-spotted spider mite, *Tetranychus telarius* (L.), in laboratory studies. Materials were applied by dip application, and the per cent control was noted 2½ days after treatment. Ethion and Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate)) were the most effective compounds of the 17 materials tested.

A number of acaricidal and insecticidal compounds have been developed for the pesticides market in recent years. Many of these materials are organophosphorous compounds. Ethion is one of these materials discovered in the laboratories of the Niagara Chemical Division, Food Machinery and Chemical Corporation, Middleport, N. Y. During the past 4 years, ethion has been evaluated extensively against several species of mites and insects (Dickinson 1958, Kurtz & Fullmer 1959). Ethion has appeared to be particularly promising as an acaricide.

Ethion was compared with 16 other commercial materials in laboratory studies. Thirteen of these materials were organophosphates. This study deals with initial acaricidal activity only, *i.e.*, the activity of these compounds against the adult two-spotted spider mite, *Tetranychus telarius* (L.). The term initial acaricidal activity is used to differentiate this type of activity from ovicidal, larvicidal, or residual activity. Per cent control was noted 2½ days after treatment.

METHODS.—Two-spotted spider mites of the green color form, *Tetranychus bimaculatus* Harvey of the *Tetranychus telarius* (L.) complex, were used in this investigation. Mites were reared on Henderson dwarf lima bush beans, *Phaseolus lunatus* L. Individually potted, bifoliate pinto bean (*Phaseolus vulgaris* L.) plants were used in all tests. Leaves from the culture table were cut into sections with approximately 50 to 70 adult mites and placed on leaves of plants to be treated. Wilting of leaf sections within 3 to 5 hours effected mite transfer.

The majority of compounds were tested as wettable powder formulations (25% active ingredient) on attaclay. Phosdrin®[3], TEPP, schradan, and demeton were tested as technical or emulsifiable concentrate formulations. Aramite®, parathion, methyl parathion, and Phosdrin consisted of Entomological Society of America Standards obtained from the Nutritional Biochemicals Corporation, Cleveland, Ohio.

Infested plants were treated by immersion of each leaf in the test suspensions for approximately 2½ seconds. Treated plants were removed to a greenhouse. Counts of the number of live and dead adult mites were made 2½ days after treatment. For each material a minimum of 2 tests using 4 concentrations and 4 replicate counts were employed. After correcting for natural mortality by Abbott's formula (Abbott 1925), LD_{50}'s with 19–20 confidence limits were determined by the method of Litchfield & Wilcoxen (1949). Toxicity indices for materials were determined as described by Sun (1950).

RESULTS AND DISCUSSION.—The LD_{50} values, confidence limits, and toxicity indices of all compounds used in this investigation appear in table 1. Ethion was used as the standard in this study. On this basis, ethion with an LD_{50} value of 0.95 p.p.m. had a toxicity index of 100. The next most effective compounds were Delnav® and Tetram®. Karathane®, with an LD_{50} of 83 p.p.m. and a toxicity index of 1.0, was the least effective. Although the majority of compounds in this study were organophosphates, it should be noted that excellent activity was obtained with two nonphosphates, Kelthane® and Aramite.

Although dip application methods were used exclusively in this study, the rapidity and accuracy obtained with this procedure facilitated testing a number of compounds. Cole & Fisk (1955) used dip application methods to check

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[3] Chemical definitions of compounds not bearing common names adopted by the Entomological Society of America are given in a list preceding "References Cited."

Table 1.—Adult two-spotted spider mites, initial activity.

COMPOUND	LD_{50} (P.P.M.)	CONFIDENCE LIMITS 19/20[a]	TOXICITY INDEX[b]
Ethion	0.95	0.89– 1.01	100.0
Delnav	1.28	1.21– 1.35	74.2
Tetram	1.55	1.43– 1.68	61.3
Demeton	3.31	3.09– 3.55	28.7
Kelthane	4.45	4.30– 4.62	21.3
TEPP	4.90	4.52– 5.30	19.4
Parathion	5.05	4.71– 5.42	18.8
EPN	5.93	5.36– 6.56	16.0
Phosdrin	6.18	5.63– 6.78	15.4
Aramite	8.75	8.07– 9.49	10.8
Methyl parathion	9.58	9.02–10.17	9.9
Phostex	10.8	10.1 –11.5	8.8
Guthion	12.8	11.9 –13.7	7.4
Diazinon	19.4	17.8 –21.2	4.9
Schradan	20.3	19.8 –20.9	4.7
Malathion	41.6	38.9 –44.4	2.3
Karathane	83.0	77.8 –88.5	1.1

[a] After Litchfield & Wilcoxen (1949).
[b] After Sun (1950).

the effectiveness of five materials on adult two-spotted spider mites. In their tests with the green form of two-spotted spider mite, LD_{50}'s of: 2.5, 2.9, 3.9, 6 and 48 p.p.m. were obtained for TEPP, Aramite, EPN, Dimite®, and malathion, respectively. Mortality counts were made either 24 or 48 hours after treatment.

The present investigation dealt with only the initial activity of a number of acaricides against the adult stage of the two-spotted spider mite. It must be considered that acaricides as a group, however, not only differ in their spectrum of activity against species other than mites, but also in their control of respective mite stages (March 1958). Excellent acaricides such as ovex (Ebeling & Pence 1954) and Tedion® (Huisman *et al.* 1955), for example, are effective primarily as mite larvicides and ovicides. It is noteworthy that Tedion is not effective against adult mites, but that it has been shown to be a highly effective mite ovarian sterilant (Meltzer & Dietvorst 1955, Batth & Davidson 1959).

The majority of the materials used in this investigation were tested in wettable powder formulations, using attaclay as a diluent. It is possible that the respective manufacturers of these materials market formulations containing diluents which would be significantly more effective than those employed in table 1. With regard to the materials tested as technical or emulsifiable liquid formulations, it has been shown that many compounds in emulsifiable liquid formulations are more active than wettable powder formulations in initial activity tests (Ebeling & Pence 1953).

Ethion and Delnav were the most active compounds in this laboratory investigation with adult two-spotted spider mites. In the field, the final test of a material, performance would be influenced by a number of factors. Some of the more important factors which have to be considered in the ultimate selection of an acaricide are: stability and residual activity, systemic and fumigative properties, effectiveness against the various stages of mites, control of resistant strains, mammalian toxicity, spectrum of activity—including effect on parasites and predators, plant safety and economics.

CHEMICAL DEFINITIONS OF PROPRIETARY COMPOUNDS REFERRED TO IN THIS PAPER

Aramite®—2-(*p-tert*-butylphenoxy)-1-methylethyl 2-choroethyl sulfite
Delnav®—2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethyl phosphorodithioate)
Diazinon® *O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate
Dimite®—4,4′-dichloro-*alpha*-methyl-benzhydrol
Guthion®—*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazine-3-(4*H*)-ylmethyl) phosphorodithioate
Karathane®—2-(1-methylheptyl)-4,6-dinitrophenyl crotonate
Kelthane®—1,1-bis(*p*-chlorophenyl) 2,2,2-trichloroethanol
Phosdrin®—1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate
Phostex®—mixture of bis(dialkyloxyphosphinothioyl)disulfides, 3:1 ratio of ethyl and 2-propyl alkyl groups
Tedion®—2,4,4′,5-tetrachlorodiphenyl sulfone
Tetram®—*O,O*-diethyl *S*-(2-diethylamino)-ethyl phosphorothioate [hydrogen oxalate salt]

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The Effect of Tedion on the Eggs and Larvae of Three Strains of the Two-Spotted Spider Mite, *Tetranychus telarius*[1]

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Abstract

Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone) had little or no contact effect on eggs of different ages from two-spotted spider mites, *Tetranychus telarius* (L.). However, a high percentage of hatching larvae were killed. Tedion-treated females produced a high percentage of nonviable eggs the first 24 hours with a marked decrease thereafter. The number of nonviable eggs produced by untreated females after feeding on Tedion residues increased with the length of feeding time. A strain of spider mites previously exposed to Tedion under greenhouse conditions was resistant to these effects.

Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone), a new acaricide, has been found to affect the larvae and nymphs of the two-spotted spider mite, *Tetranychus telarius* (L.), and the eggs in the ovaries of treated adult females (Huisman *et al.* 1955, Meltzer 1956, Ferguson 1958). A previous study at this laboratory supported these findings and provided additional information concerning the long residual effect of Tedion (Henneberry *et al.* 1960).

The present study was conducted to determine:

1. The contact effect of Tedion on eggs of different ages and hatching larvae.
2. The effect of Tedion on eggs and hatching larvae produced by:
   a. Treated adult female mites.
   b. Untreated adult female mites after feeding for different lengths of time on residues.

Materials and Methods.—Three strains of spider mites were used. The Massachusetts and Cranbury-1 strains have been characterized as susceptible and resistant, respectively, to organophosphorus acaricides (Taylor & Smith 1956). The third strain, designated Cranbury-10, originated from the organophosphorus resistant Cranbury-1 strain. Mites were isolated from the parent colony in 1950. Since that time they have been maintained on rose plants in a greenhouse at Beltsville, Md. The roses have been treated with different acaricides and acaricide combinations under greenhouse conditions. In 1958 and 1959, Aramite® (2-(*p*-(*tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite)-Tedion combinations effectively controlled these mites (Taylor & Henneberry 1959).

Lima bean seedlings, trimmed of all but the two primary leaves, were used as the host plants in all experiments. The petiole of each leaf was banded with lanolin to keep mites from wandering off the plant. Plants were set in water-filled 150-milliliter bottles and the neck of each bottle was smeared with a sticky barrier (Deadline) to prevent contamination from the benches.

A wettable-powder suspension of Tedion (1 pound of 50% Tedion per 100 gallons) was used to treat plants or mites. All experiments were conducted in a randomized-block design with four replications.

To determine the effect of Tedion on eggs in different stages of development, 20 untreated female mites were transferred from the colony to untreated bean seedlings on each of 5 successive days. Each group was removed after 24 hours of oviposition. On the fifth day, all plants were dipped in the Tedion suspension. Most of the eggs deposited on the first day had hatched to the larval stage, the rest of the eggs ranged in age from 1 to 4 days.

The effect of Tedion on eggs and larvae produced by treated mites was determined by transferring untreated females to untreated seedlings. The mites and seedlings were then dipped in the Tedion suspension. Twenty treated mites were individually removed from treated to untreated plants as soon as the plants were dry and the same mites were subsequently transferred to untreated plants two more times on successive days.

Lima bean seedlings were also treated and then infested with 80 to 100 untreated adult mites. Twenty of the mites were then transferred from the treated plants after 1, 24, or 48 hours to untreated plants for 24 hours. Living and dead mite forms were counted 7 days after treatment in all experiments.

Results.—Tedion applied to eggs of the three mite strains had little toxic effect since the majority of them hatched. The hatching larvae of the Cranbury-1 and Massachusetts strains were susceptible. The hatching larvae of the Cranbury-10 strain were only slightly affected by the Tedion treatment, but significantly more were susceptible when the mites were in the larval stage at the time of treatment (table 1).

A high percentage of the eggs produced by Tedion-treated mites of the Cranbury-1 and Massachusetts strains on untreated bean plants for 24 hours were nonviable. When the same mites were transferred to clean plants on 2 successive days, the egg mortality decreased (table 2). The eggs of the Cranbury-10 strain were not affected in this manner.

Mites allowed to feed on residues of Tedion for 1 hour, and transferred to untreated plants, produced viable eggs. However, increasing the length of feeding time of the Massachusetts and Cranbury-1 strain likewise increased the number of nonviable eggs. Eggs deposited by the Cranbury-10 strain were viable regardless of the amount of feeding. A low mortality of the hatched larvae indicates that the effect does not extend beyond the egg stage when the progeny is not exposed to Tedion (table 3).

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Table 1.—Effect of Tedion applied to eggs of three different strains of two-spotted spider mites.

Age of Eggs When Treated (days)	Per Cent Mortality	
	Eggs	Larvae[a]
	Massachusetts	
1	2	97 a
2	2	95 ab
3	5	95 ab
4	5	93 ab
5[b]	10	85 ab
	Cranbury—1	
1	5	81 b
2	5	93 ab
3	7	91 ab
4	8	91 ab
5[b]	7	90 ab
	Cranbury—10	
1	8	9 d
2	6	11 d
3	5	15 d
4	4	19 d
5[b]	10	42 c

[a] Means in a column not followed by the same letter are significantly different.

[b] Mostly hatched at the time of treatment.

Table 2.—Effect of three different strains of two-spotted spider mite eggs and larvae from females treated with Tedion and placed on untreated bean plants.

Days After Treatment	Per Cent Mortality	
	Eggs[a]	Larvae
	Massachusetts	
1	98 a	1
2	73 b	0
3	5 d	0
	Cranbury-1	
1	98 a	1
2	56 c	1
3	15 d	2
	Cranbury-10	
1	11 d	1
2	3 d	1
3	1 e	2

[a] Means in a column not followed by the same letter are significantly different.

Table 3.—Effect on three different strains of two-spotted spider mite eggs and larvae from females not treated by Tedion but placed for different lengths of time on Tedion residues.

Length of Feeding Time (hours)	Per Cent Mortality	
	Eggs[a]	Larvae
	Massachusetts	
1	7 a	1
24	48 b	3
48	82 d	2
	Cranbury-1	
1	11 a	0
24	65 c	6
48	76 cd	4
	Cranbury-10	
1	3 a	1
24	14 a	3
48	13 a	1

[a] Means in a column not followed by the same letter are significantly different.

COMMERCIAL AND EXPERIMENTAL ORGANIC INSECTICIDES

By Eugene E. Kenaga

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Laboratory and Field Tests of New Insecticides for Grasshopper Control[1]

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Abstract

New insecticides were evaluated for grasshopper control on rangeland in Western United States. The most effective of laboratory-screened compounds were field tested on 1½-acre plots with ground equipment or on 40- or 640-acre plots with aircraft. The most promising compounds from the field tests were Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl)phenyl phosphorothioate), Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate), Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), dimethoate, General Chemicals 3707 (1,3-bis(methoxycarbonyl)-1-propen-2-yl dimethyl phosphate), malathion, Monsanto CP-7769 (hexaethyl (ethylthiomethylidyne) triphosphonate), phosphamidon, and Sevin® (1-naphthyl methylcarbamate). A discussion of the techniques of evaluating new insecticides through testing in the laboratory and in small- and large-scale plots is presented.

Grasshoppers are extremely important pests of range forage in the western natural-grass States. Research has not yet developed any satisfactory method of control other than insecticides. Some chlorinated hydrocarbons have proved highly effective in preventing grasshopper damage to range forage. Two of these, aldrin and heptachlor, have been widely used with excellent effectiveness and economy but are no longer generally recommended. Their application produced undesirable residues on range forage and more particularly in the fat of animals grazed thereon (Entomology Research Division 1959). As a result there is an urgent need for insecticides which can be safely used on forage.

In searching for substitute insecticides certain basic facts must be considered in addition to their ability to kill grasshoppers. Toxic residues must degrade to nontoxic substances either at the site of application or within the animal which has ingested treated forage. The low per-acre forage value of most western rangeland precludes the use of many insecticides economically feasible on high money-value crops. Insecticides highly toxic to mammals and feasible in small-scale operations present a considerable hazard to man, domestic animals, and wildlife when used over the thousands of acres often treated by aircraft for grasshopper control. In addition, stability during storage, in sunlight and under various temperatures; resistance to hydrolysis; and residual toxicity of the insecticide to the insect itself must be considered. Laboratory and field tests reported herein were conducted during the years 1957–59 to evaluate new materials, particularly organophosphorus compounds, with aldrin as a standard.

Laboratory Tests.—*Materials.*—Compounds screened in the laboratory were of technical grade as received from the manufacturer or the Agricultural Research Service. Those used in the field studies were formulated by the manufacturer. The compounds referred to in this paper are as follows:

Aldrin
Am. Cyanamid 12008 (*O,O*-diethyl *S*-isopropylthiomethyl phosphorodithioate)
Bayer 22684 (*O*(2-chloroethyl 2,2-dichlorovinyl methyl phosphate))
Bayer 24869 (1-chloro-2-propyl 2,2-dichlorovinyl ethyl phosphate)
Bayer 25141 (*O,O*-diethyl *O*-(*p*-methylsulfinyl)phenyl phosphorothioate)
Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate) (also known as Baytex)
Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate)
Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate)
dieldrin
dimethoate
1-Fluorochlordene (1-fluoro-4,5,6,7,8,8-hexachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoindene)
General Chemicals 3561 (dimethyl 2-(1,1,3,3-tetrafluoro-2-hydroxy)-propyl-phosphonate)
General Chemicals 3583 (2-chloro-1-(2,5-dichlorophenyl)vinyl diethyl phosphate)
General Chemicals 3661 (1,3-bis(methoxycarbonyl)-1-propen-2-yl diethyl phosphate)
General Chemicals 3707 (1,3-bis(methoxycarbonyl)-1-propen-2-yl dimethyl phosphate)
General Chemicals 4072 (2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate)
Hercules AC 5727 (*m*-isopropylphenyl methyl carbamate)
Kepone® (decachloroöctahydro-1,3,4-metheno-2*H*-cyclobuta [cd] pentalen-2-one)
malathion
Monsanto CP-7769 (hexaethyl (ethylthiomethylidyne)triphosphonate)
phosphamidon
Sevin® (1-naphthyl methylcarbamate)
Shell SD 3562 (dimethyl 1-(dimethylcarbamoy)-1-propen-2-yl phosphate)
Shell 4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanoisobenzofuran)
toxaphene

Methods.—The laboratory screening tests were designed to eliminate compounds that are ineffective at practical dosages and to give a starting point for field dosages. The tests did not establish any critical order of effectiveness but did aid in the selection of the most promising from a large number, with field performance the ultimate consideration. Some compounds tested were new and all were of unknown insecticidal activity to grasshoppers. The toxicities shown by feeding tests and by topical application were not always in agreement; however, their relationship to the standard was valid and in most cases the dosages established in the laboratory were in general agreement with field results.

Either adults or fifth-instar nymphs of the two-striped grasshopper (*Melanoplus bivittatus* (Say)) reared in the laboratory from field-collected eggs were used. The egg

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pods were kept in storage at 40° F., then placed in moist sandy soil in plaster-of-Paris chambers 12×6½×4 inches, and held at a constant temperature of 85° for hatching. Glass lids, painted black, excluded light. The newly hatched nymphs were collected in glass vials or tubes inserted into holes in the hatching chamber. The nymphs were then transferred to plastic tubes, 18 inches long and 3 inches in diameter, with screen caps on each end, and reared to the third instar at 85°. At this stage the nymphs were transferred to 24×24×18 inch screen cages and held at room temperature to complete development. Fresh romaine lettuce and wheat bran were fed daily.

Room temperatures were supplemented with a 250-watt infrared bulb placed at one side of the cage and burned for 8 hours each day. The position of the bulb furnished a temperature gradient within which the grasshoppers could orient themselves. For *M. bivittatus* the optimum temperature was 87° to 90° F. Cages and tubes were sterilized with a 1:9 solution of sodium hypochlorite after each use. The method usually produced the first adults in about 24 days, with a survival of 60% to 75% from an initial population of approximately 300 grasshoppers.

Two methods of testing the compounds were employed. In early 1957 grasshoppers were sprayed with emulsions or solutions, depending on the compound, at the rate of 1 gallon per acre. The spray unit was adapted from a dusting chamber described by Pepper & Hastings (1943) to simulate low-volume application by aircraft. An open sheet-metal tank 28.3 inches in diameter and 13 inches in depth was mounted on a belt-driven turntable and revolved within a hood equipped with an air-exhaust system. The inside of the tank was lined with clean wrapping paper before each treatment. A DeVilbiss hand-atomizer nozzle, adjustable vertically, was mounted above the center of the spray chamber. A 4-ml. glass vial was substituted for the standard reservoir and the intake was constricted by the insertion of a fine-glass capillary tube which was set to remove an exact amount of fluid from the vial. The constricted intake permitted continuous spraying of amounts as low as the equivalent of 1 gallon per acre while the drum made several revolutions. Compressed air was supplied by a small motor-driven diaphragm pump connected to the atomizer nozzle through a pressure regulator. Pressure was maintained at 10 p.s.i. and sprays were emitted in small droplets with a minimum of mist. After treatment the grasshoppers, usually in groups of five, were removed, confined in screen cages, and fed untreated lettuce. Mortalities were compared with those from an aldrin standard of 2 ounces per acre.

In the second method, used in 1958–59, technical insecticides were dissolved in a suitable solvent, usually acetone, and applied to the first two abdominal sternites of the grasshoppers or to lettuce discs for oral tests. The applications were made with a 0.25-ml. tuberculin syringe fitted with a 27-gauge hypodermic needle and driven by a micrometer screw (Trevan 1922). Different dosage levels were tried and each toxicity was compared with that of recrystallized aldrin at approximately the LD-50 level. After treatment, the grasshoppers were caged with untreated lettuce as food and mortalities were determined at 24, 48, and 72 hours.

Results.—A total of 78 compounds were screened in the laboratory during the period 1957–59. Most of the compounds produced little or no mortality when tested and were eliminated from further consideration. Of the 78 compounds screened, 19 were considered suitable for further investigation when available in sufficient quantities. Results of the screening tests with these compounds are shown in table 1.

Table 1.—Results of laboratory screening of new insecticides against the two-striped grasshopper. Bozeman, Montana, 1957–59.

Insecticide[a]	Dosage (μg./insect)	Mortality in Hours Shown (%)		
		24	48	72
Topical application				
Aldrin (Standard)	4.25	83	100	100
	2.125	33	58	59
Bayer 22684	34.0	100	100	100
	17.0	73	83	83
Bayer 24869	34.0	100	100	100
	8.5	92	92	92
Bayer 25141	17.0	100	100	100
	8.5	66	66	66
Bayer 29493	34.0	50	67	67
	17.0	33	33	33
Dibrom	4.25	100	100	100
	2.125	83	83	83
Dimethoate	4.25	100	100	100
	2.125	0	17	17
1-Fluorochlordene	4.25	100	100	100
	2.125	33	33	50
General Chemicals 3561	4.25	100	100	100
	2.125	17	33	33
General Chemicals 3661	8.5	100	100	100
	4.25	87	87	87
General Chemicals 3707	4.25	87	100	100
	2.125	50	50	50
General Chemicals 4072	8.5	100	100	100
	4.25	50	50	50
Hercules AC 5727	34.0	100	100	100
	17.0	83	83	83
Kepone	68.0	0	17	50
	34.0	0	0	33
Monsanto CP-7769	34.0	100	100	100
	8.5	33	83	83
Phosphamidon	34.0	100	100	100
	17.0	75	83	83
Shell SD 3562	34.0	100	100	100
	17.0	33	33	33
Aldrin (standard)[c]	2	100	100	100

Table 1 continued from preceding page.

Insecticide[a]	Dosage (μg./insect)	Mortality in Hours Shown (%) 24	48	72
Am. Cyanamid 12008[b]	4	100	100	100
	2	60	63	63
Diazinon	8[c]	100	100	100
	4[c]	90	90	90
	2[b]	77	87	87
Sevin[b]	4	50	50	50
	4	20	20	33
Oral application				
Aldrin (standard)	4.25	50	60	70
Bayer 22684	8.5	10	10	10
Bayer 25141	8.5	50	50	50
Dimethoate	8.5	100	100	100
	4.25	50	50	50
1-Fluorochlordene	8.5	100	100	100
	4.25	64	73	73
General Chemicals 3561	2.125	60	60	60
General Chemicals 3707	2.125	10	10	10
General Chemicals 4072	4.25	33	33	33
Hercules AC 5727	8.5	50	50	50
Kepone	34.0	0	33	75
Monsanto CP-7769	8.5	0	0	0

[a] Acetone solution unless otherwise indicated.
[b] Emulsion.
[c] Kerosene solution.

SMALL-PLOT TESTS.—*Methods.*—Insecticides were selected for field trials mainly from those showing promise in the laboratory tests. However, several compounds were tested in the small plots without laboratory screening on the basis of manufacturers' recommendations. The insecticides selected were first tested on 1½-acre plots located on short-grass Montana rangeland near Three Forks in 1958, and Harlowton in 1959. The dominant grasshopper species at both locations were *Aulocara elliotti* (Thos.), *Amphitornus coloradus* (Thos.), *Melanoplus bilituratus* (Walk.), *M. packardii* Scudd., *M. infantilis* Scudd., *Bruneria brunnea* (Thos.), and *Ageneotettix deorum* Scudd.

The insecticides were divided into groups of two or three, with aldrin as a standard at 2 ounces per acre. Most of the treatments were replicated six or seven times, when sufficient material was available, with untreated check plots included in each replicate. The insecticides were applied in diesel oil at the rate of 1 gallon of solution per acre. The treatments were made with a truck-mounted turbine blower-type sprayer fitted with a USDA air-broadcast nozzle which produced a 30-foot swath with droplet size simulating that obtained with aircraft. The plots were separated by 30-foot aisles, and each treatment was applied at random and related to an adjacent check plot. Thirty samples, each consisting of the grasshoppers caught in one stroke of a net, were taken on each plot before and 1 and 3 days after treatment. Sampling was done at right angles to the spray swaths. Mortalities were calculated by an adaptation of Abbott's formula used by Hinman & Cowan (1947) and Connin & Kuitert (1952). An increase of 5% or more in mortality at 3 days was considered evidence of effective residual toxicity. Any reduction in mortality at 3 days indicated failure of the insecticide to kill grasshoppers infiltrating from the surrounding grassland.

In the 1958 small-plot field tests the formulation of Sevin (36M) was a mayonnaise-type emulsion of microfine particles suspended in mineral oil and surfactants. Settling occurred in this formulation and considerable stirring was required before application. In the 1959 tests the formulation of Sevin (85P) was a microfine oil-dispersible powder which was more suitable for use in diesel oil. Dibrom, dimethoate, and Bayer 25141 formulations were neither miscible nor emulsifiable in diesel oil but were kept in suspension through bypass agitation. Satisfactory application of these formulations in diesel oil could not be expected if applied in quantity by aircraft in control-type operations. Shell SD-3562 was received as technical material which was readily soluble in diesel oil. All of the other insecticides were formulated as concentrates emulsifiable in diesel oil.

Results.—Small-plot tests were conducted with 16 insecticides selected on the basis of their performance in the laboratory screening tests, their availability, and information furnished by the manufacturers. Results of these tests are given in table 2.

Residual toxicity was definitely shown by Sevin, Monsanto CP-7769, Shell 4402, Bayer 25141, phosphamidon, and dimethoate. Residual toxicity was indicated by Bayer 24869 and Hercules 5727, but there were too few replicates using these materials for positive conclusions. Judging from the high mortalities from the use of Bayer 29493, a lower dosage might be expected to give good grasshopper control. In the limited tests with a new formulation of malathion, SF 59, good initial kills were obtained at 8 and at 16 ounces per acre, but there was no indication of a continued kill of grasshoppers that moved onto the plots during the next 3 days.

Several miscellaneous small-plot tests were conducted during late July and August of 1959. Dimethoate was applied to 5-acre plots at dosages of 1, 2, and 3 ounces per acre by means of the same procedures used in the replicated tests. Insect mortality from the 1-ounce dosage increased from 66% at 1 day after treatment to 92% 13 days after treatment, that from the 2-ounce dosage increased from 69% to 93%, and that from the 3-ounce dosage from 91% to 94%. The formulation 85P of Sevin applied to a 5-acre plot at 8 ounces per acre gave a nearly 100% kill of late-instar nymphs at the end of 4 days. A month later a similar application against adults infesting dry, mature vegetation gave a kill of 86% after 4 days.

Adult grasshoppers were caged over sprayed vegetation in batches of 50 per cage and allowed to feed for 3 days. Seven days after treatment 3 ounces of dimethoate per acre had killed 51%, 16 ounces of malathion (SF 59)

Table 2.—Results of small-plot tests with various insecticides for grasshopper control, Montana, 1958–59.

Insecticide[a]	Toxicant (oz./a.)	No. of Replications	Per Cent Mortality After— 1 Day	3 Days
Experiment 1 (1958)				
Malathion	8	7	77	77
	16	7	86	87
Sevin (36M)	8	7	62	81
	16	7	77	85
Aldrin (standard)	2	7	81	89
Experiment 2 (1958)				
Dibrom	6	6	89	80
General Chemicals 3707	2	6	89	83
Am. Cyanamid 12008	4	6	87	91
Aldrin (standard)	2	6	83	90
Experiment 3 (1958)				
Monsanto CP-7769	4	6	77	86
	8	6	89	92
Shell 4402	2	6	83	92
	4	6	95	95
Aldrin (standard)	2	6	92	97
Experiment 4 (1958)				
Bayer 25141	1.5	7	74	80
	2	6	72	80
Bayer 22684	2	4	61	57
	2.5	6	63	59
Bayer 24869	2	2	6	41
	2.5	2	36	61
Aldrin (standard)	2	7	73	78
Experiment 5 (1959)				
Phosphamidon	4	6	47	71
	8	6	74	89
Dimethoate, soluble concentrate	2	6	71	91
	4	6	82	97
Aldrin (standard)	2	6	81	91
Experiment 6 (1959)				
Hercules AC 5727	4	3	66	65
	8	6	74	77
	12	3	85	90
Bayer 29493	16	6	98	99
	24	6	98	99
Aldrin (standard)	2	6	93	99
Experiment 7 (1959)				
Shell SD 3562, technical	4	1	92	89
	8	1	95	97
	16	1	98	95
Gen. Chemicals 3583, oil concentrate	6	1	72	81
	8	1	81	81
	10	1	90	79
Aldrin (standard)	2	1	81	81
Experiment 8 (1959)				
Malathion (SF 59)	8	3	92	81
	16	2	97	90
Aldrin (standard)	2	2	90	93

[a] Applied in No. 2 diesel oil at 1 gallon per acre. Emulsion concentrate unless otherwise indicated.

per acre had given no kill, and 8 ounces of Sevin per acre had killed 16%. Vegetation was also mature and dry at the time of these tests.

LARGE-SCALE TESTS.—*Methods.*—The large-scale tests included medium-sized 40-acre and large-sized 640-acre plots. The 40-acre plots were utilized after the small 1½-acre plots to further establish dosage rates. Insecticides were applied with small aircraft of the Piper Cub-Aeronca class. The 640-acre plots were utilized after the dosage rate was established to test the materials under simulated-control campaign conditions. Insecticides were applied with TBM and Twin Beech aircraft, employed in Federal-State-rancher cooperative campaigns. Flagmen indicated the flight line and spacing of spray strips on all of the 40-acre plots. On the 640-acre plots only the corners were marked with flags. Flight height over the medium-sized plots was generally under 20 feet but varied from 50 to 100 feet over the large ones. All insecticides were premixed at the flight strip and applied in 1 gallon of diesel fuel per acre.

Counts of live grasshoppers were made by the pointer method on all plots prior to treatment, at 1, 3, and 7 days following treatment on the 40-acre plots, and at 3 and 7 days on the 640-acre plots. Kills were expressed as a per cent reduction using pretreatment and posttreatment counts. In general, all treatments were replicated three times in all tests.

Plots selected for treatment were located in several areas of Montana and California and represented various types of short- and medium-grass rangeland. Vegetation varied from good to very poor, weedy, overgrazed stands. The dominant grasshopper species were approximately the same as in the small-plot tests.

In 1957 Diazinon, along with malathion at 1 pound per acre, was tested at 8 ounces per acre in Montana and 8 and 6 ounces in California on 40-acre plots. In 1958 malathion was further tested in Montana at ¾ pound per acre on 640-acre plots and in 1959 at 1 pound per acre on both 40- and 640-acre plots. In addition, in 1959 dimethoate, Sevin, General Chemicals 3707, Bayer 25141, Dibrom, and Monsanto CP 7769 were evaluated in Montana on 40-acre plots as was Sevin on 640-acre plots. Aldrin at 2 ounces per acre was the standard in all tests.

Results.—Results obtained from the 40-acre plots are given in table 3, and from the 640-acre plots in table 4. The dieldrin test at one-half ounce per acre (table 4) was included in the event that this very low dosage would eventually be shown in a more favorable light than other hydrocarbons, from the residue standpoint.

In these tests no differences between treatments were apparent after 7 days, with the exception of malathion at three-fourths pound per acre. All other dosages were selected to give control, and the results were not unexpected. Since 2 ounces of dimethoate and 4 ounces of Sevin (table 3) gave somewhat lower 24-hour kills, these were probably their minimum effective dosages under the conditions of the tests. The minimum dosage for malathion appeared to be 1 pound per acre.

DISCUSSION.—Laboratory screening tests eliminated compounds which did not give a high enough kill of grasshoppers at practical dosages when compared with aldrin as a standard. Through information supplied

Table 3.—Results of aerial spraying of 40-acre plots[a] with various insecticides for grasshopper control, Montana and California. 1956–59.

Insecticide[b]	Toxicant (oz./a.)	Average Mortality (%) After Days Shown 1	3	7
Dimethoate, soluble concentrate	2	71.7	97.1	98.9
	4	86.9	99.5	99.9
Sevin (85P)	4	65.1	92.9	96.4
	8	69.5	95.0	97.7
Malathion	16	91.6	99.1	99.6
General Chemicals 3707	2	89.8	98.3	99.0
Bayer 25141	2	83.6	97.4	99.4
Dibrom	6	87.4	97.5	98.4
Monsanto CP-7769	8	86.3	98.4	99.7
Diazinon	8[c]	—	—	90.8
	6[d]	—	—	97.7
Aldrin (standard)	2	85.9	99.2	99.7

[a] 3 replications unless otherwise indicated.
[b] Emulsion concentrate unless otherwise indicated.
[c] 4 replications.
[d] 2 replications.

Table 4.—Results of aerial spraying of 640-acre plots[a] with emulsion concentrates of various insecticides for grasshopper control, Montana 1957–1959.

Insecticide	Toxicant (oz./a.)	Per Cent Mortality in 7–10 Days Range	Average
Malathion	16	81–99	92
	12	44–55	50
Sevin (85P)	16	99–99	99
	8	99–99	99
Toxaphene	24[b]	67–99	88
Dieldrin	0.5	99–99	99
Aldrin	2[c]	93–99	98

[a] 3 replications unless otherwise indicated.
[b] 4 replications.
[c] 6 replications.

largely by the manufacturer the mammalian toxicity, formulation, and physical and chemical properties of the most promising compounds were considered in relation to grasshopper control. Small-plot tests established field dosages and furnished needed information on formulations, speed of kill, and residual action. Further tests of the most promising compounds were made by large and small aircraft as a final evaluation. Before a compound is recommended for grasshopper control tests are made to determine whether it produces undesirable residues on the forage or in meat and milk. Whether or not it then becomes commercially available depends upon the manufacturer.

Efficient grasshopper control on rangeland is dependent on residual as well as inherent toxicity of the insecticide. Those insecticides with good residual toxicity provide a distinct advantage in that grasshoppers receiving sublethal dosages or missed entirely during application will ingest lethal amounts as they move about from day to day. This advantage is particularly important when aircraft are used since cost is kept at a minimum by low-gallonage application and coverage is not always as good as might be desirable. The insecticide should retain its chemical stability long enough to kill that portion of the population not eliminated by direct contact, without creating a residue problem for grazing livestock.

Under a variety of climatic conditions an insecticide in No. 2 diesel oil is more efficient than in water, particularly when applied by aircraft. Formulations that can be used with relatively nonvolatile oils are preferable because they allow less evaporation, which can result in crystallization or in a reduction of the volume of solution reaching the ground. They also show better adherence to vegetation and to the insects, and their penetration is facilitated. Insecticides that depend almost entirely on initial contact and feeding during the first day leave little margin for error in application and usually require a higher dosage rate and complete coverage to insure control.

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Loss of Parathion and DDT to Soil from Aqueous Dispersions and Vermiculite Granules[1]

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ABSTRACT

Both P^{32}-parathion and C^{14}-DDT were strongly adsorbed by soil from water dispersions. From two different volumes of water, the percentage loss of DDT to soil in 24 hours was the same (78%), but the loss of parathion with the larger volume was about half (38%) that with the smaller volume. Bioassays of extracts of water and soil with larvae of *Anopheles quadrimaculatus* Say showed no appreciable loss in toxicity of parathion in 24 hours. Tests with marsh water showed that large differences in pH, total solids, and chloride content had little effect on the distribution and codistillation of DDT at 0.02 p.p.m. Parathion was released into water readily from vermiculite granules; decreasing the concentration of parathion in the granules or increasing the depth and volume of water increased the percentage released at a given time. With vermiculite-DDT granules less than 20% of the DDT was found dispersed in the water at both 2 and 24 hours, which indicated that it was sloughed off at the time of application.

Field and laboratory studies have demonstrated the effectiveness of granular formulations of insecticides for the control of mosquito and sand fly larvae (Whitehead 1951, Keller *et al.* 1953, LaBrecque & Goulding 1954, Chapman 1955, LaBrecque *et al.* 1956, Weidhaas 1957). Mosquito larvae have been controlled by the introduction of solubilized insecticides into irrigation water (Gahan *et al.* 1955a, b, Gahan & Mulhern 1955, Gahan 1957, Weidhaas *et al.* 1959). However, little is known about the behavior of the toxicants in these formulations when applied for insect control. Among the many factors involved are: (1) the fate of the toxicant in water, (2) the influence of soil; (3) the rate of release of the toxicant from the granular vehicle into water; and (4) the manner by which the insect acquires a lethal dose. Any information regarding these complex interrelated factors would provide a better understanding of these formulations and their application for mosquito control.

The availability of samples of P^{32}-labeled parathion (34.1 μc./mg.) and C^{14}-labeled DDT (2.1 μc./mg.) made possible a study of the fate of these materials in water dispersion over soil when applied in different formulations. Radiometric analyses were performed in all tests, and corrections for self-absorption and decay made where applicable.

TESTS WITH WATER SOLUTIONS OR SUSPENSIONS.—Previous laboratory experiments using bioassay techniques have shown that the toxicity to mosquito larvae of insecticides dispersed in water is reduced by the presence of soil (Weidhaas *et al.* 1959). The processes causing the loss of toxicity were investigated by determining the distribution of parathion and DDT in water over soil. A sandy soil was collected locally, screened through a 10-mesh sieve, and over-dried at 110° C. The organic content was found to be 11.25% (dry weight) by loss on ignition. A pH of 4.36 was obtained when 100 ml. of distilled water was mixed with 50 grams of the dried soil.

The tests were made with 50 grams of soil in glass jars 76 mm. in diameter. The soil was moistened with 25 ml. of untreated water, and then covered with a filter paper disk to prevent agitation of the soil when more water was added. In the tests with parathion, sufficient water treated with an acetone solution of the insecticide was then added to the jars to give a total of 100 or 250 ml. of water containing 0.02 p.p.m. of toxicant. Changes in the insecticide content of the supernatant water and of the soil were followed by analyzing benzene extracts made immediately and 6 and 24 hours after the solutions were prepared. Since previous bioassay tests have shown no loss in toxicity in the presence of filter paper without soil, indicating that the paper adsorbed little parathion, the soil and filter paper were extracted together. In the tests with DDT sufficient untreated water was added to give a total of 100 or 250 ml. and 0.02 p.p.m. of DDT was added in acetone solution and the test jars were sealed to prevent the loss of insecticide by codistillation with water in a process described by Bowman *et al.* (1959). Isooctane extractions of the supernatant water were made after 0, 6, and 24 hours. The sides of the jars were rinsed with isooctane to remove any DDT adhering to the glass and the rinses added to the extractions. The amount of DDT in the soil was obtained by subtraction. The results are given in table 1.

Table 1.—Percentage of DDT and parathion in soil and supernatant water over a 24-hour period.[a]

HOURS AFTER PREPARATION	WATER		SOIL	
	DDT	Parathion	DDT	Parathion
	100-ml. volume			
0	81	89	19	9
6	44	47	56	47
24	22	8	78	63
	250-ml. volume			
0	94	84	6	9
6	60	71	40	21
24	23	44	77	38

[a] 0.02 p.p.m. in two volumes of water.

Immediately after preparation of the solutions or suspensions most of the parathion or DDT was found in the water; but the amount in the soil increased, as time elapsed, which indicated that both these insecticides were adsorbed. At 6 and 24 hours, the per cent of parathion lost to the soil from the smaller volume of solution was greater than that from the larger, but the actual weight lost was greater from the larger volume. With DDT the same percentage was lost in 24 hours from both volumes.

After 24 hours the total recovery of parathion was low.

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A mixture of benzene and ethanol improved the extractions from the soil, but 5% to 10% still could not be recovered. Even in a similar test using 10 times the amount of parathion (50 μg.) an average of only 95% was recovered. Recoveries were the same from sealed as from open jars, which indicated that no parathion was lost by volatilization or codistillation with water and that the unrecovered toxicant was bound in the soil and unextractable with the solvents employed.

To determine whether parathion had been detoxified in the presence of soil, bioassays of extracts from soil and water were made with fourth-instar larvae of *Anopheles quadrimaculatus* Say. Radiometric analyses showed the total amounts of parathion plus any derivatives in both extracts, but the extracts were tested for toxicity on the assumption that the total radioactivity represented unchanged parathion. Comparison with standard solutions indicated that the parathion extracted from water solution after 24 hours showed no loss in toxicity, whereas that extracted from the soil showed no loss in toxicity in one test and only 20% loss in another. Any derivatives formed were therefore equal in toxicity to parathion and no detoxication had occurred.

DISTRIBUTION OF DDT IN BRACKISH MARSH WATER.—In laboratory tests using distilled water, Bowman *et al.* (1959) and Weidhaas *et al.* (1960) showed that, in very dilute suspensions, DDT is heterogeneously dispersed and lost by codistillation and these two factors influence larval mortality. Since DDT has been used as a larvicide for the control of some of the salt-marsh mosquitoes, a study was made of its distribution in and loss from brackish marsh water. Analyses of samples of water collected from four marshes on the Florida east coast are given below:

Marsh	*pH*	*Total Solids (p.p.m.)*	*Chloride (p.p.m.)*
1	7.00	2,790	750
2	7.48	8,730	2,260
3	8.81	17,580	4,800
4	7.35	3,945	1,050

Suspensions of DDT (250 ml. of 0.01 p.p.m.) were made with water from marshes 1 and 3, which had the lowest and highest values. The percentage distribution of the DDT at 25±0.5° C. in closed jars was as follows:

	2 Minutes		*24 Hours*	
	Marsh 1	*Marsh 3*	*Marsh 1*	*Marsh 3*
Upper half of suspension	37.2	33.1	27.6	28.8
Lower half of suspension	35.6	32.4	24.0	26.2
Upper third of exposed walls	12.8	16.1	16.9	14.9
Lower two-thirds of walls and bottom	10.1	6.6	13.8	10.1
Total recovery	95.7	88.2	82.3	80.0

The losses of DDT by codistillation from open jars containing water from marshes 1 and 3 in 24 hours, determined at the same time as the distribution of the insecticide, were 59.6% and 68.3%. The percentages were not the same because the loss of water from the marsh-1 sample was 17.9 grams and that from the marsh-3 sample 19.8 grams. The respective rates of loss were 0.0885 and 0.0918 mg./ml. Both the distribution and loss were similar to that reported previously by Bowman *et al.* The large differences in pH, total solids, and chloride content had little effect on the distribution or loss of DDT by codistillation at 0.01 p.p.m.

From studies at 5 p.p.m. Hawkins & Kearns (1956) postulated that the toxicity of DDT in aqueous dispersions to *quadrimaculatus* larvae was decreased because of flocculation and consequent settling. Flocculation was presumed to be influenced by *zeta* potential and, consequently, by ion concentrations. The data presented above indicate that these factors would not be active in dispersions in the order of 0.01 p.p.m. Weidhaas *et al.* (1960) have shown that the toxicity of DDT dispersions to larvae of *quadrimaculatus* was correlated with its heterogeneous dispersion and codistillation.

TESTS WITH VERMICULITE GRANULES.—Tests were made to determine whether the treatment of water with a floatable granular formulation would reduce the adsorption of parathion by the soil. Formulations on vermiculite granules were prepared at parathion concentrations of 0.01% and 0.025% (wt./wt.) by adding the required amount of radioactive parathion in acetone to 5 grams of granules. The mixture was stirred while the solvent was evaporated with an air stream. Twenty mg. of each formulation was sprinkled on the surface of 100 and 250 ml. of water standing over 50 grams of soil so as to give a calculated concentration of 0.02 p.p.m. After 24 hours only 20% of the parathion was found in either volume of water, an indication that the residual concentration in the water could not be increased by the use of floatable granules.

It was considered possible that the release of parathion from these formulations might have been rapid but that adsorption was not retarded. To test this possibility, the release of parathion from 20 mg. of a 0.025%-vermiculite formulation into 250 ml. of water (0.02 p.p.m.) in a 76-mm.-diameter glass jar was measured at 6 and 24 hours and found to be complete. In a previous study using chemical analyses, release of parathion in 24 hours from 1% and 10% granular formulations with concentrations equivalent to 1 and 20 p.p.m. was shown to be incomplete (Weidhaas 1957). Since the latter concentration approaches the maximum solubility of parathion in water, the rate of release appeared to be influenced by the concentrations in the granules, and possibly the volume of water into which the granules were placed. Additional tests were made with a 1% formulation on vermiculite in the absence of soil. When 20 mg. were applied to different volumes of water, the amount of parathion found in the water after 24 hours was 55% in 0.2 liter, 64% in 2 liters, and 77% in 20 liters. These and previous data indicate that the concentration of parathion in the water, as well as that in the granular material, influenced the rate of release.

The effect of the depth of water standing over soil on the distribution and adsorption of parathion from treated vermiculite granules was also studied. Ten milligrams of 1% granules was added to water standing over one-half inch of soil in two sets of battery jars, 152.5 mm. in diameter. In one set the water was 8 inches deep (3,650 ml.) and in the other only 1 inch (460 ml.). The concentration of parathion in the water at 6 and 24 hours is shown below:

	P.p.m.	*Per Cent*
8 inches		
6 hours	0.016	58
24 hours	.026	92

1 inch		
6 hours	.12	56
24 hours	.10	55

If all the parathion were released from the granules and none adsorbed by the soil, the concentration in the water would be 0.22 p.p.m. in the 1-inch, and 0.028 in the 8-inch depth. At 6 hours only about half these amounts were present at both depths, and the concentration was eight times greater in the 1-inch than in the 8-inch depth. However, at 24 hours the concentration in the shallow water had not increased over that found at 6 hours and was only 3.8 times greater than in the deeper water. The depth of water had a pronounced effect on adsorption by the soil.

The release of DDT from vermiculite granules in the absence of soil was also studied. Twenty milligrams of granules containing 0.035% of DDT, prepared in the same manner as the parathion granules, was placed on 250 ml. of water in jars. The jars were sealed to prevent loss of the insecticide by codistillation. At 2 hours 17% and at 24 hours 16% of the DDT was found in the water. This finding indicated that the insecticide was probably sloughed off the granules when they were first placed in the water and that no further amount was released, a reasonable assumption since DDT is very hydrophobic and highly insoluble in water.

DISCUSSION.—Radiometric data and bioassay tests with parathion reported in this paper confirm the results of previous studies by Weidhaas *et al.* (1959) that insecticides dispersed in water lose toxicity in the presence of soil, and that this loss results from adsorption on the soil. Both parathion, which is water-soluble at the concentration used, and DDT, which is nearly insoluble, were adsorbed readily.

In the studies with vermiculite granules, parathion was almost completely released into water, but only a small percentage of DDT was found in the water, an indication that it was sloughed off as fine particles or with fine granular particles. Although the mechanism of release of toxicants from granules did not appear to be complex, the amount released and present in water at any given time after application depends on (1) their solubility in water, (2) the concentration of toxicant in granules, (3) the rate of application, (4) their proximity to soil and the total amount of water as determined by depth, (5) the type of carrier, and (6) agitation of water.

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Insecticidal Control of the Sugar-Beet Root Maggot and Yield of Sugar Beets[1]

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Abstract

Two years' results in Manitoba have shown that heptachlor applied at 0.5 to 2 pounds per acre to the seed furrow on ammonium phosphate (11-48-0) reduced the sugar-beet root maggot, *Tetanops myopaeformis* (Roed.), about 80%. Some 20% of the maggots appear to tolerate this 4-fold increase in rate of heptachlor applied. This fact may represent an initial tolerance because heptachlor-fertilizers were used only 1 year prior to the tests reported. Sugar beet yields were increased 1 to 2.4 tons per acre by favoring the production of larger beets, but sugar content was not affected. However, yield was not increased when heptachlor was added at 1 pound per acre to the seed furrow as granules and sidedressed with fertilizer, although effective maggot control was obtained. Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) applied at 1 pound per acre to the seed furrow on ammonium phosphate (11-48-0) effectively reduced maggots but was phytotoxic. With the same formulation and rate, phorate and Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) were not effective.

A direct bioassay did not detect residues of heptachlor, Diazinon, phorate or Trithion in harvested beets. Only a trace (0.01 p.p.m.) of heptachlor epoxide was detected chemically.

Seed treatments with heptachlor, Diazinon, phorate and Trithion were less effective for maggot control and did not increase the yield of sugar beets.

It was shown previously (Allen *et al.* 1959) that heptachlor or aldrin-fertilizer mixtures applied in the seed furrow to give 1 pound of toxicant per acre gave effective control of the sugar-beet root maggot, *Tetanops myopaeformis* (Roed.). The reduction in the number of maggots appeared to be associated with an increase in yield, when beet stands were at least 75% complete.

This paper reports control tests completed at Altona, Manitoba, in 1958 and 1959, with heptachlor and several organic phosphate insecticides. The method of formulation and application of these insecticides are considered in relation to larval control, beet stand, size and yield of beets.

Materials and Methods.—Insecticides used for soil and seed treatments were heptachlor, phorate, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate), and Trithion® (*S*-(*p*-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate). For soil treatments fertilizer mixtures were prepared with heptachlor 72% technical, phorate 44% on carbon powder, Diazinon 90% technical and Trithion 50% flowable. For seed treatments heptachlor 40% on talc, phorate 44% on carbon powder, Diazinon 25% wettable and Trithion 50% flowable were applied to seed (Allen *et al.* 1961). Tables 1 and 2 show the formulations, methods and rates at which the actual toxicants were applied in 1958 and 1959.

Plots were arranged in randomized blocks replicated eight times; each plot consisted of four 60-foot rows. Details were given previously on formulation of the fertilizer mixtures, method and rate of seeding, seed treatment with captan and the application of fertilizer (Allen *et al.* 1961).

The maggot infestations were estimated from each plot in mid-September by counting the maggots in 10 soil samples, 8″ by 8″ and 12″ deep. Each sample was taken from the soil directly beneath a beet in the outside rows. In 1959 the beets taken out with each sample were cleaned and weighed to determine size at that time. Each randomized block was sampled in succession.

The yield and number of beets were determined in early October. Two 50-foot areas from the central rows of each plot were harvested and the beets were washed before weighing. Percentage sugar was determined by the Manitoba Sugar Company.

The data on number of maggots per beet, yield, beet number and size were examined by analysis of variance and the multiple range test (Duncan 1955).

In 1958 and 1959 preliminary bioassays were completed after harvest for beets treated with insecticides at 1 or 2 pounds per acre (table 3). The direct method of Sun & Pankaskie (1954) was used. *Drosophila melanogaster* Meig. was cultured on cornmeal-molasses-agar medium at 80 ± 1° F. and all tests were completed at this temperature. Twenty-five beets from treated plots were washed well, quartered and one-quarter from each beet was diced and the sample composited. Five-pound samples were selected and put in polyethylene bags for storage at 40° F. To each 150 grams of beet tissue, 100 ml. of water was added and the tissue macerated. Standards were prepared by adding insecticides at four dosage levels to untreated beets before they were macerated. About 20 grams of macerated beet was placed in 6-ounce jars; 10 male and 10 female, 2-day old CO_2 anaesthetized flies were introduced on paper trays. For each lot of insecticide-treated beets, five samples of beet tissue were assayed. Percentage mortality was calculated by totalling the number of dead flies and those unable to stand. Dosage mortality data were analyzed by the method of Litchfield & Wilcoxon (1949) and the 95% confidence interval of 0.2 LD_{50} was taken as a measure of sensitivity (Sun & Sun 1952) of the method for each insecticide.

In 1959, chemical analyses were completed on heptachlor-treated beets by Velsicol Chemical Corp., Chicago. The samples of beet tissue were divided, one portion was bioassayed and the remainder was analysed for hepta-

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Table 1.—Effect of insecticide, formulation and method of application on average number of root maggots and yield of sugar beets at Altona, Manitoba, 1958.

INSECTICIDES	TOXICANT (OZ./A.)	NUMBER OF		WEIGHT PER BEET AT HARVEST (LB.)	YIELD (TONS/A.)
		Maggots per Beet[b]	Beets per 100 Feet of Row		
Soil treatment[a]					
Heptachlor 1.0%	12.8	4.1±1.0 [c]	80.0	1.5	13.1
1.0%	16	3.8±0.9	70.1	1.8 [d]	13.4
1.25%	16	4.5±0.9	73.0	1.8	13.9 [d]
1.25%	16	4.3±1.0	77.5	1.7	13.9
Diazinon 1.25%	16	3.1±0.9	58.2 [d]	2.1	12.7
Phorate 1.25%	16	10.2±2.5	65.4	1.8	12.3
Seed treatment					
Heptachlor 40%	1	10.8±2.2	83.1	1.4	12.6
Diazinon 25%	1	11.1±2.3	75.9	1.6	13.0
Phorate 44%	1	18.8±3.2	79.4	1.6	14.0
Untreated (fertilizer)	—	19.5±3.7	78.4	1.5	12.1

[a] Details on formulation of fertilizer mixtures as shown Allen *et al.* (1961, table 4).
[b] Mean and 95% confidence interval for 80 beets.
[c] Means not connected by the same vertical line significantly different at 1% level (Duncan 1955).
[d] Means connected by a vertical line significantly different from means of untreated plots at 5% level.

chlor by the method of Ordas *et al.* (1956) and heptachlor epoxide was determined by a tentative method (Velsicol Corp. 1960).

RESULTS AND DISCUSSION.—*Root Maggot Reduction.*—In 1958 and 1959 soil treatments with heptachlor applied as fertilizer mixture (tables 1 and 2) to give 1 pound of toxicant per acre reduced the number of maggots about 78% and 80%, respectively. The method of applying heptachlor to the seed furrow (table 2) did not alter the effectiveness of this insecticide. The result was the same when it was applied as 20% granules or on fertilizers constituted with various solvents (table 1). It is remarkable that dosages of 0.5, 1, 1.5 and 2 pounds per acre of heptachlor applied on fertilizer all gave essentially the same result suggesting that some 20% of the maggots tolerated a 4-fold increase in heptachlor. Furthermore the 95% confidence limits (tables 1 and 2) show that effective insecticides consistently reduced the number of maggots to a low level. This preventive method was designed to treat the soil area surrounding the beets where the egg clutches are laid. Direct evidence of insecticide tolerance is required because we do not know the proportion of young larvae that survive treatment and whether or not the insects are able in some way to avoid the treated area. Heptachlor-fertilizer mixtures were first used for sugar-beet root maggot control in 1957.

Table 2.—Effect of insecticide, formulation and method of application on average number of root maggots and yield of sugar beets at Altona, Manitoba, 1959.

INSECTICIDES	TOXICANT (OZ./A.)	NUMBER OF		WEIGHT PER BEET (LB.)		YIELD (TONS/A.)
		Maggots per Beet[a]	Beets per 100 Feet of Row	September	October	
Soil treatments[b]						
Heptachlor 2.5%	32	5.8±1.4 [c]	85.0	1.3 [d]	1.4 [d]	12.4 [d]
1.87%	24	5.2±1.5	84.7	1.3	1.4	12.7
1.25%	16	6.3±1.3	87.6	1.3	1.3	12.3
0.62%	8	5.9±1.3	84.2	1.4	1.3	11.9
Heptachlor[e] 1.25%	16	5.1±1.0	89.9	1.2	1.3	12.4
Heptachlor[f] 20%	16	6.6±1.4	92.0 [d]	1.1	1.1	11.2
Trithion 1.25%	16	19.4±3.6	81.9	1.2	1.3	11.4
Untreated (fertilizer)		29.6±4.5	79.1	1.0	1.2	10.3
Seed treatment						
Heptachlor 40%	1	14.4±3.3	84.5	1.2	1.2	11.2
	2	19.1±3.1	87.1	1.3	1.2	11.7
Trithion 50%	1	21.0±3.8	83.1	1.2	1.3	11.4
	2	20.0±3.4	80.7	1.2	1.3	11.0
Phorate 44%	1	22.2±3.4	80.7	1.2	1.2	10.7
Untreated (fertilizer)	—	24.8±4.0	79.7	1.2	1.2	10.2

[a] Mean and 95% confidence interval for 80 beets.
[b] Details on formulation of fertilizer mixtures as shown Allen *et al.* (1961, table 4).
[c] Means not connected by the same vertical line significantly different at 1% level (Duncan 1955).
[d] Means connected by a vertical line significantly different from means of untreated plots at 5% level.
[e] On triple superphosphate, sidedressed with nitrogen.
[f] Granulated insecticide, sidedressed with ammonium phosphate.

Table 3.—Residues of insecticides determined by bioassay and chemical analyses of sugar beets grown in soil treated with fertilizer mixtures.

Treatment	Toxicant (lb./a.)	Bioassay: Per Cent Mortality 1958	Bioassay: Per Cent Mortality 1959	Sensitivity[a] (p.p.m.)	Chemical Analyses 1959: Heptachlor (p.p.m.)	Chemical Analyses 1959: Heptachlor Epoxide (p.p.m.)
Heptachlor	1.0	0.0		0.12–.17		
	1.0	1.0	1.0	0.12–.17	0.01	0.02
	1.0[b]		1.0	0.12–.17	0.01	0.02
	2.0		2.0	0.12–.17	0.01	0.02
Diazinon	1.0	0.0		0.29–.37		
Phorate	1.0	1.0		0.17–.30		
Trithion	1.0		0.0	1.2 –1.9		
Untreated		0.0	0.0		0.01	0.01

[a] 95% confidence interval of 0.2 LD_{50}.
[b] Applied to seed furrow as 20% granules.

Applied as fertilizer mixtures to give 1 pound of toxicant per acre Diazinon and phorate reduced maggots 84% and 48%, respectively, but both were phytotoxic (Allen *et al.* 1961). Trithion was not effective.

In 1958 heptachlor and Diazinon seed treatments at 1 ounce per acre reduced maggots 46% and 43%, respectively. Phorate was not effective. In 1959 heptachlor and Trithion at a 1- or 2-ounce rate and phorate at the 1-ounce rate were not significantly different in number of maggots from untreated plots.

Insecticide Residues.—Preliminary direct bioassay with *Drosophila melanogaster* (table 3) showed that beets treated with heptachlor, Diazinon, phorate or Trithion at 1 pound per acre did not contain detectable residues. In 1959 chemical analyses for heptachlor and heptachlor epoxide supported bioassay evidence that residues were low and only trace quantities of heptachlor epoxide were indicated.

Sugar Beet Yield and Root Maggot Reduction.—Data presented in table 1 show that heptachlor applied in fertilizer mixtures at rates of 0.8 to 1 pound per acre increased yields 1 to 2 tons per acre and significantly reduced the number of maggots. However, the increase in yield was significant at the 5% level for only two of the treatments, and one gave significantly larger beets at harvest. For Diazinon and phorate the increase in beet size resulted from phytotoxicity that significantly altered the stand of beets at harvest.

Data given in table 2 show that heptachlor applied in fertilizer mixtures at rates of 0.5 to 2 pounds per acre increased the yield of beets 1.6 to 2.4 tons per acre. There was a definite association between the number of maggots and beet size measured in mid-September in relation to yield. This was not as clearly shown at beet harvest. Yield was not increased significantly when heptachlor at 1 pound per acre was applied to the seed furrow as granules and fertilizer was side dressed. When heptachlor was similarly applied on triple superphosphate yield was increased but not beet size. A Trithion-fertilizer mixture did not reduce the number of maggots or increase yield.

Seed treatments (tables 1 and 2) with heptachlor, Diazinon and Trithion were not effective in reducing the number of maggots and did not increase the yield of beets. The response to heptachlor seed treatments has not been consistent, although increased yields were previously reported by Gojmerac (1957) and Allen *et al.* (1959). Phorate appeared to increase yield, without causing a reduction of maggots in 1958, but this was not true in 1959.

We conclude that in Manitoba, heptachlor-fertilizer applied to the seed furrow to give 0.5 to 1 pound of toxicant per acre gave effective maggot control. The treatment usually resulted in a significant increase in yield, but the increase in beet size may not always be significant. Diazinon, phorate and Trithion were either too phytotoxic or ineffective and did not increase the yield. The same was true of seed treatments with heptachlor, Diazinon, phorate and Trithion which are ineffective.

It is noteworthy that, for the untreated plots in 1959, the correlation between the number of maggots and weight per beet in mid-September was −0.184, significant at the 1% level.

However, many agronomic factors affect the growth of sugar beets and there is evidence that yield is influenced by the type of fertilizer, its placement (Schmehl *et al.* 1952), or the stands established after thinning (Eckroth and Cormany 1957). Accordingly, in spite of effective maggot control, a fertilizer effect may account for the fact that heptachlor applied as granules in the furrow, with ammonium phosphate sidedressed, did not increase the yield; whereas the same quantity of insecticide similarly applied on triple superphosphate, sidedressed with nitrogen gave a significant increase in yield.

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Effect of Insecticide Fertilizer Mixtures and Seed Treatments on Emergence of Sugar Beet Seedlings[1]

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ABSTRACT

Two years' results in Manitoba have shown that heptachlor-fertilizer mixtures, applied to the seed furrow for control of the sugar-beet root maggot, *Tetanops myopaeformis* (Roed.), did not reduce sugar beet stands significantly. Phytotoxic effects were not serious, even under dry soil conditions, when fertilizer mixtures (consisting of 1.25% actual heptachlor 1.8% to 5.0% xylene on ammonium phosphate (11-48-0)) were applied to the seed furrow at 80 pounds per acre. The phytotoxic effect of insecticide fertilizer mixtures was attributed to fertilizer which interferes with the uptake of the water required by the seed for germination, or to insecticide or solvent which may cause seedling damage. Phorate and Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) were more phytotoxic than Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate) or heptachlor in field and laboratory tests. Similarly the insecticide solvent Velsicol AR-60 shown by this laboratory test to be more phytotoxic than xylene depressed seedling emergence significantly in the field. Laboratory tests showed that when soil moisture was 21% or 16.6%, seedling emergence was reduced significantly when heptachlor-fertilizer mixtures or fertilizer were applied in contact with the seed. But the addition of heptachlor and 2% solvent did not depress emergence more than fertilizer alone. Seed treatments with heptachlor and Trithion or phorate and Diazinon were not phytotoxic when used in conjunction with captan-treated seed. Without captan, heptachlor and phorate depressed seedling emergence, but heptachlor in combination with captan may enhance the protective action of captan. Captan-treated seed improved seedling stands slightly when used with heptachlor-fertilizer mixtures.

It has been shown that insecticide-fertilizer mixtures give effective control of the sugar-beet root maggot, *Tetanops myopaeformis* (Roed.), in North Dakota (Callenbach *et al.* 1957, Gojmerac 1957) and in Manitoba (Allen *et al.* 1959). Such treatment decreases seedling emergence and these reductions become of concern when complete beet stands are not obtained after careful thinning. The importance of determining the reasons for these phytotoxic effects will increase as the sugar beet industry adopts precision planting and mechanical thinning to obtain uniform stands. The advantages of these cultural procedures may not be achieved if root maggot damage or adverse growing conditions early in the season are aggravated by phytotoxic effects.

With fertilizer mixtures the insecticide, solvent or fertilizer may contribute to the phytotoxic effect. The germination of sugar beet seed and emergence of seedlings is affected by soil moisture (Hunter & Dexter 1950, Hunter & Erickson 1952), particularly when mineral fertilizers are applied to the drill row (Snyder 1957, Dubetz *et al.* 1959). Insecticide-fertilizer mixtures prepared with chlorinated hydrocarbon insecticides were found less phytotoxic than those prepared with organic phosphate insecticides (Gojmerac 1956, 1957). However the type and quantity of insecticide solvent (Guyer & Hutson 1956) used in such formulations have not been extensively considered.

This paper reports on phytotoxicity tests completed at Altona, Manitoba in 1958, when rainfall was low during the period of seedling emergence, and in 1959 when soil moisture was adequate. Laboratory and field studies were combined to determine the influence of insecticides and the type and quantity of solvents on seedling emergence. The importance of soil moisture in relation to phytotoxic effects of the components of the insecticide-fertilizer

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mixture is discussed. Further work on the effect of insecticide-fertilizer mixtures and seed treatments on control of the sugar-beet root maggot in relation to sugar beet yield is reported by Allen *et al.* (1961).

MATERIALS AND METHODS.—*Laboratory Tests on Phytotoxicity.*—The method of Casida & Allen (1951) was used to evaluate the relative phytotoxicity of certain insecticide solvents and insecticides used in prepared fertilizer mixtures. The solvents emulsified with phosphate buffer and tested were xylene, heavy aromatic naphtha, Velsicol AR-60 and Velsicol AR-50G. Solvent concentrations were 100, 200 and 500 p.p.m. The insecticides tested as xylene emulsions were heptachlor 72% technical, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) 90% technical, phorate 90% technical, and Trithion® (*S*-(*p*-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate) 50% flowable, which contained additional solvent and emulsifier. They were added to 100 p.p.m. of xylene in concentrations of 25, 50, 75 and 100 p.p.m. actual insecticide. Insecticide-fertilizer mixtures have insecticide to solvent ratios approximating 1 to 2.

Sugar beet seed was soaked 2 hours in water and germinated between moist blotting paper, in darkness at 76° F. After 72 hours seeds having primary roots about 10.0 mm. in length were selected and measured. Eight seedlings were transferred to filter paper in 9-cm. petri dishes containing 5.0 ml. of solvent-phosphate-phosphate buffer pH 6.0, in which the specified amounts of insecticide-solvent or solvent were emulsified with 0.05 mg. of saponin. The phosphate buffer 0.025 M. and saponin, as used for controls, gave better root growth at pH 6.0 than at pH 5.0 or pH 7.0. The insecticides and solvents did not change pH. Duplicate tests were completed with the solvents and insecticides. In each test, duplicate determinations were made at each concentration. After 48 hours incubation at 76° F. the roots were measured. Phytotoxicity was evaluated, by observing general root appearance and by comparing percentage increase in root length or phytotoxicity indices (Casida & Allen 1951).

The effect of soil moisture on seedling emergence in relation to the application of fertilizer was evaluated under controlled conditions in a plant growth chamber. Soil moisture was maintained at two levels for the 12-day period of seedling emergence. The daily cycle of plant growth conditions were, 16 hours under lights at 70±1° F., relative humidity 60±2%; 8 hours dark at 60±2° F., relative humidity 50±3%. The seeds were planted in 1-gallon crocks and plastic cones were placed over the soil surface to minimize evaporation. The containers were weighed daily and water was added through an opening in the apex of the cone to maintain a constant weight. The openings were plugged with cotton and a layer of silica sand prevented crusting of the soil surface.

The soil obtained from the experimental area at Altona, Manitoba had the following mechanical composition: 62.6% sand (2.0–0.5 mm.), 19.8% silt (0.5–0.002 mm.) and 17.6% clay (less than 0.002 mm.). Soil moisture constants are expressed as percentage moisture on a basis of soil dried to constant weight at 105° F.; the 95% confidence limits of determination errors are shown. Percentage moisture at field capacity, determined by the method of Lehane & Staple (1953) was 24.3±0.8%. Permanent wilting percentage was 10.4±0.4% (Lehane & Staple 1951). Accordingly at field capacity the amount of moisture readily available for plant growth was 14.0%.

Soils were prepared that contained 21% and 16.6%, which is 76% and 45% respectively, of the moisture available in respect to this soil at field capacity. Moisture was determined on each lot of soil after it was moistened and equilibrated and after each test was finished. Two tests were completed at these moisture levels and the differences between tests or during each test did not exceed ±1.0% and ±0.6%, respectively.

In each test the following treatments were replicated three times at each moisture level: fertilizer, 1.25% heptachlor-fertilizer mixture containing 2.0% xylene and 1.25% heptachlor-fertilizer mixture containing 2.0% heavy aromatic naphtha. The fertilizer, ammonium phosphate (11-48-0) was applied at a calculated rate of 80 pounds per acre. Controls contained no fertilizer.

One-gallon crocks were filled with moistened soil and 24 seeds were planted directly above the fertilizer in a circular row, 4 inches in diameter at a depth of three-quarter inch. To prevent damping-off seed was treated with captan at a rate of 6 ounces actual per 100 pounds of seed and 5 p.p.m. was added to the dry soil. Emerged seedlings were counted daily and the mean emergence period in days calculated (Leach *et al.* 1946).

Field Tests on Phytotoxicity.—In table 4 the rates of insecticide application, the quantities of insecticide and solvent used in formulation, and also the type and placement of fertilizer are indicated.

For fertilizer mixtures the insecticides used were heptachlor 72% technical, phorate 44% on carbon powder, Diazinon 90% technical and Trithion 50% flowable. Solvents used for heptachlor-fertilizer mixtures were xylene, and heavy aromatic naphtha. For comparison a heptachlor formulation that contained no solvent was prepared. Fertilizer was treated with 40% heptachlor on talc prepared by grinding heptachlor 72% technical with talc and 1.0% Tamol. No additional solvent was added when Diazinon 90% and Trithion 50% flowable were sprayed on fertilizer or phorate 44% on carbon powder was used to coat fertilizer.

For seed treatments the same insecticides were applied to seed at the rate of 1 pound of toxicant per 100 pounds of seed. The quantities of heptachlor 40% on talc and 25% Diazinon wettable powder required were mixed with each pound of seed and 10.0 ml. of 1.0 per cent Methocel until all the powder was taken up. Seed was coated or sprayed with phorate 44% on carbon powder or 50% Trithion flowable, respectively.

Plots were arranged in randomized blocks replicated eight times, each plot consisted of four 60-foot rows; outside guard rows were provided. An International 4-row beet drill equipped with a V-belt metering device calibrated for precise delivery was used to apply seed and fertilizer. Processed commercial seed treated with 8.0 ounces of 75% captan per 100 pounds was sown at 6.0 pounds per acre. The seed and fertilizer whether treated or not treated were drilled into the furrow. In control plots untreated seed and fertilizer were used. Ammonium phosphate (11-48-0) was applied at 80 pounds per acre.

Table 1.—Effect of insecticide solvents on average root growth of sugar beet seedlings exposed 48 hours at 76° F.

Solvents	Increase in Root Length (%) Concentration of Solvents (p.p.m.) 100	200	500	Phytotoxicity Index[a]	
Velsicol AR-60	45	15	11	88.6	[b]
Heavy aromatic naphtha	30	49	19	84.2	
Velsicol AR-50G	76	62	16	74.8	
Xylene	139	144	155	28.1	
None	203				

[a] Per cent inhibition of growth caused by solvent treatment.
[b] Means not connected by the same vertical line significantly different at 1% level (Duncan 1955).

Table 2.—Effect of insecticide, emulsified in xylene (100 p.p.m.), on average root growth of sugar beet seedlings exposed 48 hours at 76° F.

Insecticides	Increase in Root Length (%) Concentration of Insecticide (p.p.m.)[a] 25	50	75	100	Phytotoxicity Index[b]	
Diazinon	85	43	36	26	73	[c]
Phorate	68	65	78	41	64	
Heptachlor	122	83	110	86	43	
Trithion	159	148	176	182	4	
None (Xylene)	173					

[a] Difference necessary for significance between concentration, at 5% level 13.0.
[b] Per cent inhibition of growth caused by insecticide treatment.
[c] Means not connected by the same vertical line significantly different at 1% level (Duncan 1955).

When Triple superphosphate (0-45-0) was applied to the furrow, ammonium nitrate (0-21-0) was applied as a sidedressing to give nitrogen and phosphate equivalent to 80 pounds of 11-48-0. When granulated heptachlor was placed in the furrow ammonium phosphate (11-48-0) was sidedressed at 80 pounds per acre.

In 1958 the effect of various amounts of the solvents used in fertilizer mixtures was tested on seedling emergence. Xylene, heavy aromatic naphtha and Velsicol AR-60 at 1%, 2% or 5% by weight were added singly or in combination with 1.25% heptachlor to ammonium phosphate (11-48-0). At the 5% concentration talc was added to absorb excess solvent. Plots were arranged in randomized blocks replicated four times; each plot consisted of one 60-foot row. The method and rates of seeding and fertilizer application were those outlined above. For this test the captan-treated seed and untreated seed were taken from the same seed lot. The plots were seeded on May 21 and seedling emergence was slow because of insufficient soil moisture. From June 4 to 16 no rain fell and the soil became dry and crusted. On June 16 soil moisture was measured at the surface and seed level as this would represent the minimum present during the interval of seedling emergence.

The seedling stand on each plot was measured immediately before the beet thinning operation by counting the number of beet-containing inches in 4, 100-inch lengths of row. The beets were thinned to one per foot of row and stand was again evaluated, to establish whether or not significant differences in the initial stand remained.

The beet stand was finally evaluated at harvest by recording the number of beets per 100 feet of row. The data were examined by analysis of variance and the multiple range test (Duncan 1955).

RESULTS.—*Laboratory Test on Phytotoxicity.*—The effect of various solvents on root growth are shown in table 1. Xylene inhibited root growth to some extent. Heavy aromatic naphtha and Velsicols AR-60 or AR-50G were significantly more toxic and the differences between the low and high concentrations of these solvents were shown to be significant ($P=0.05$). At the high concentrations these solvents practically stopped growth and turned the root and cotyledons dark brown or black. When technical insecticides were combined with xylene (table 2) Diazinon and phorate were more toxic than heptachlor. Trithion, in the commercial formulation used, was not more toxic than xylene alone. The effects of Diazinon and phorate increased with concentration. At 75 and 100 p.p.m. the former distorted the roots causing them and the cotyledons to turn dark brown; the latter similarly discolored the roots. Roots and cotyledons grown in contact with Trithion and xylene were healthy but some roots were slightly browned by heptachlor at all concentrations.

The effect of soil moisture on seedling emergence in relation to application of fertilizer or heptachlor fertilizer mixtures is shown in table 3. When the available moisture was 45% and 76% seedling emergence was not affected in the absence of fertilizer. In contact with fertilizer emergence was delayed and significantly reduced at both moisture levels. The reduction of 85% at 45% available moisture was serious. The addition of heptachlor and the solvents did not delay or depress emergence more than fertilizer alone.

Field Tests on Phytotoxicity.—Seasonal conditions of rainfall and soil moisture influence greatly the germination of beet seed and seedling emergence. Accordingly it is under conditions of low moisture that the placement and quantity of fertilizer applied and other phytotoxic effects may combine to affect beet stand. In 1958 rainfall was deficient in May and June amounting to 0.27 and 0.77 inches; for the corresponding months in 1959 the amounts were 4.26 and 2.86, respectively. Table 4 shows that the stand before thinning in 1959 was about twice that in 1958 and that one plant per foot remained after thinning. However, in 1958 Diazinon and phorate fertilizer mixtures significantly reduced seedling emergence and the differences in stand were not removed by thinning. In 1959 fertilizer mixtures applied to give 1.5 or 2.0 pounds of heptachlor per acre reduced emergence significantly but with ideal growing conditions complete stands were obtained after thinning. Seedling emergence decreased as heptachlor content increased. Trithion, in the formulation used, was not phytotoxic.

The phytotoxicity of Diazinon and phorate fertilizer mixtures is attributed to the insecticide content (table 2) as solvents were not used in these formulations. In 1958

Table 3.—Average number of sugar beet seedlings from 24 captan-treated seeds in 12 days, and mean emergence period.

Formulation	Available Soil Moisture[a]			
	76%		45%	
	No. of Plants Emerged[b]	Mean Emergence in Days	No. of Plants Emerged[b]	Mean Emergence in Days
1.25% heptachlor+2% xylene on ammonium phosphate (11–48–0)	22.5 [c]	7.8	10.5 [c]	9.1
1.25% heptachlor+2% aromatic naphtha on ammonium phosphate (11–48–0)	23.6	7.7	5.7	9.5
Ammonium phosphate (11–48–0)	24.0	7.6	5.0	8.9
None	35.3	5.7	33.8	6.2

[a] Soil moisture 21% and 16.6% of dry soil weight, respectively.
[b] Potential emergence was 1.4 per seed.
[c] Means not connected by the same vertical line significantly different at 1% level (Duncan 1955).

heptachlor-fertilizer mixtures containing 2.2% xylene or 1.7% heavy aromatic naphtha did not affect seedling emergence more than a fertilizer mixture prepared with 40% heptachlor on talc, in which no solvent was used, or untreated fertilizer. In 1959 heptachlor-fertilizer mixtures containing 1.8% to 2.5% xylene were not phytotoxic until the actual heptachlor content exceeded 1.25%. Seedling emergence was not affected when 20% granulated heptachlor or 1.25% heptachlor on triple superphosphate were applied to the seed furrow and ammonium phosphate or ammonium nitrate, respectively, were applied as sidedressing.

Table 4.—Effects of insecticides, formulations and method of application on average number of beet-containing inches at Altona, Manitoba.

Insecticide	Toxicant (oz./a.)	Formulation	Per Cent Solvent	Beet-Containing Inches per 100″ of Row	
				Before Thinning	After Thinning
		Fertilizer Mixtures (1958)			
Diazinon	16	1.25% on $NH_4H_2PO_4$ (11–48–0)	None[a]	8.5 [b]	5.6 [c]
Phorate	16	1.25% on $NH_4H_2PO_4$ (11–48–0)	None[d]	8.8	5.5
Heptachlor	12.8	1.0% on $NH_4H_2PO_4$ (11–48–0)	2.2% xylene	14.8	6.8
	16	1.0% on $NH_4H_2PO_4$ (11–48–0)	2.2% xylene	11.8	6.5
	16	1.25% on $NH_4H_2PO_4$ (11–48–0)	1.7% heavy aromatic naphtha	13.0	6.4
	16	1.25% on $NH_4H_2PO_4$ (11–48–0)	None[e]	14.2	7.4
		Seed Treatments (1958)			
Diazinon	1.0	25%, wettable powder		13.5	6.9
Phorate	1.0	44%, on carbon powder		14.1	7.1
Heptachlor	1.0	40%, on talc		14.6	7.2
Untreated		$NH_4H_2PO_4$ (11–48–0)		14.1	6.9
		Fertilizer Mixtures (1959)			
Heptachlor	32	2.5% on $NH_4H_2PO_4$ (11–48–0)	2.5% xylene	16.4 [b]	7.4 [c]
	24	1.87% on $NH_4H_2PO_4$ (11–48–0)	1.8% xylene	18.5	8.0
	16	1.25% on $NH_4H_2PO_4$ (11–48–0)	1.8% xylene	19.2	8.2
	8	0.62% on $NH_4H_2PO_4$ (11–48–0)	1.8% xylene	20.5	8.1
	16	1.25% on triple superphosphate (0–45–0)[f]	1.8% xylene	24.1	7.9
	16	20% granulated insecticide	None[g]	25.4	8.2
Trithion	16	1.25% on $NH_4H_2PO_4$ (11–48–0)[h]		22.6	7.9
Untreated		$NH_4H_2PO_4$ (11–48–0)		26.1	8.2
		Seed Treatments (1959)			
Heptachlor	1.0	40% on talc		22.1 [b]	7.9 [c]
	2.0	40% on talc		20.2	7.9
Trithion	1.0	50%, flowable[h]		20.7	8.0
	2.0	50%, flowable[h]		19.4	7.9
Phorate	1.0	44% on carbon powder		18.7	7.9
Untreated		$NH_4H_2PO_4$ (11–48–0)		23.5	8.4

[a] Fertilizer sprayed with technical Diazinon 90%.
[b] Means not connected by the same vertical line significantly different at 1% level (Duncan 1955).
[c] Same as [b] but at 5% level.
[d] Fertilizer coated with phorate (Thimet 44-D).
[e] Fertilizer coated with 40% heptachlor on talc (heptachlor technical 72% ground in talc and 1.0% thamol).
[f] Side dressed with ammonium nitrate (21-0-0) to give nitrogen and phosphate equivalent to 11-48-0.
[g] Sidedressed with ammonium phosphate (11-48-0).
[h] Fertilizer or seed sprayed with 50% Trithion flowable.

Table 5.—Effects of heptachlor and solvents in combination with fertilizer on sugar beet stand at Altona, Manitoba 1958.

Treatments[a]	Solvent and Per Cent	Beet-Containing Inches per 100' of Row		
		Captan[b]		No Fungicide
1.25% heptachlor on fertilizer	1 Xylene	22.5	23.0	11.1
	2			
	5	19.5		
	1 Aromatic naphtha	15.5	19.6	11.5
	2			
	5	15.3		
	1 Velsicol-AR-60	17.8	17.0	12.2
	2			
	5	8.3		
No heptachlor (fertilizer)	1 Xylene	26.4	21.7	14.8
	2			
	5	24.2		
	1 Aromatic naphtha	22.2	24.9	16.1
	2			
	5	18.0		
	1 Velsicol AR-60	17.2	15.9	9.3
	2			
	5	11.2		
Controls (fertilizer)	None	25.7		17.3

[a] Ammonium phosphate (11-48-0) applied at 80 pounds per acre.
[b] Treated with 8 ounces of 75% captan per 100 pounds of seed.

Seed treatments with Diazinon and phorate at 1 ounce or Trithion and heptachlor each at 1 or 2 ounces per acre, with 80 pounds of fertilizer, were nonphytotoxic (table 4).

Data given in table 5 show the effect of solvents at 2% and heptachlor at 1.25% on seedling emergence from captan-treated seed and untreated seed. During the period of emergence in 1958 little rain fell and none was recorded from June 4 to 17. The soil dried, the surface crusted and seedling emergence was delayed. Table 6 shows that the surface soil was excessively dry and soil moisture at the seed level approached the wilting level. Rainfall from June 17 to 27 moistened the soil and brought on further emergence. Under these conditions captan increased emergence significantly (table 7) in the controls. The main treatments, consisting of solvents at 2% and heptachlor at 1.25% or solvents alone on ammonium phosphate (table 5) significantly reduced emergence from seed treated with captan or untreated seed. Heptachlor did not reduce emergence more than the solvents at 2% but there was a significant difference between solvents. The multiple range test established that Velsicol AR-60 reduced emergence more than xylene or heavy aromatic naphtha ($P=0.01$), irrespective of whether or not the seed was treated with captan. However, with all solvents captan increased average emergence about 10% in comparison with the increase in the controls.

When the solvents were added to 1.25% heptachlor-fertilizer or to fertilizer alone (tables 5 and 8) at 1%, 2% or 5%, differences between solvents and concentration levels were significant, for captan-treated seed. Velsicol AR-60 was again more phytotoxic, particularly at the 5% concentration. Heptachlor appeared to aggravate the effect of the solvents when they exceeded 2% concentration.

The protective effect of captan in relation to insecticide seed treatments is shown in table 9. Heptachlor and phorate reduced seedling emergence below that of untreated seed. However, when the seed was treated with captan heptachlor appeared to increase emergence while phorate continued to depress it. These insecticides differed significantly in this response but they did not increase or decrease emergence significantly in relation to seed not treated with insecticide. The effect of the phorate seed treatment was similar at Altona (table 4) in 1959.

DISCUSSION.—The phytotoxic effects that result in reduced sugar beet seedling emergence, after the application of insecticide-fertilizer, may be considered from two aspects: first, fertilizer may hinder or retard the imbibition of the water required by the seed for germination by increasing the osmotic effect of the soil solution. This

Table 6.—Percentage soil moisture at Altona, June 16, 1958.

Replicates	Percentage Soil Moisture	
	Depth in Inches	
	0–0.25	0.25–1.25
1	2.74±0.90[a]	13.9±1.6[b]
2	3.48±2.62	11.7±2.0
3	2.74±1.08	10.3±1.3
4	2.51±0.67	10.1±1.4

[a] Mean and 95% confidence interval for five samples.
[b] Mean and 95% confidence interval for 18 samples.

Table 7.—Analysis of variance on seedling emergence, beet-containing inches per 100 inches of row, solvent concentration 2%.

Source	Degrees of Freedom	Mean Squares	F	P
Controls				
Captan treated vs. untreated	1	411.50	23.20	<.01
Controls vs. main treatments	1	412.27	23.25	<.01
Main treatments				
Heptachlor vs. none	1	22.69	1.28	n.s.
Solvents	2	95.96	5.41	<.01
Captan vs. none	1	742.61	41.88	<.01
Replicates	3	98.75	5.57	
Error[a]	62	17.73		

[a] Interactions not significant error pooled.

Table 8.—Analysis of variance on seedling emergence, beet-containing inches per 300 inches of row, from captan-treated seed.

Source	Degrees of Freedom	Mean Squares	F	P
Heptachlor vs. none	1	1,050.35	5.24	.05
Solvents	1	3,780.01	18.86	<.01
Levels	2	1,267.39	6.32	<.01
Replicates	3	320.45	1.60	n.s.
Error[a]	60	200.34		
Total	68			

[a] Interactions not significant error pooled.

Table 9.—Effects of insecticide seed treatments and the fungicide captan on emergence of sugar beet seedlings at Gretna, Manitoba. 1959.

Insecticide (1 oz./A.)	Beet-Containing Inches per 100′ of Row	
	No fungicide	Captan
Heptachlor	7.0 [a]	33.2[b]
Phorate	6.6	21.6
Untreated	10.8	27.9

[a] Means not connected by the same vertical line are significantly different at the 5% level (Duncan 1955).
[b] Difference necessary for significance at 5% level, 8.0.

effect becomes more critical, when soil moisture tension is increased by lower soil moisture, when fertilizers with greater solubility are used, when the application rate of fertilizer is increased or when fertilizer is placed close to the seed. Secondly, the addition of insecticide or solvent may inhibit root growth or injure root tissues and retard plant growth.

The former aspect has been substantiated for sugar beet seed by Hunter & Erickson (1952), Stout *et al.* (1956), Snyder (1957) and Dubetz *et al.* (1959). A significant result of the present paper is that the addition of 1.25% heptachlor and 2% of the solvents xylene or heavy aromatic naphtha to ammonium phosphate (11-48-0), applied in contact with the seed, did not reduce seedling emergence more than the fertilizer alone, even when available soil moisture was only 45%. Furthermore a comparison of years characterized by low and high rainfall shows that a satisfactory beet stand can be established, by careful thinning, even under dry conditions. In field planting the seed and fertilizer are well mixed with soil and are not in direct contact.

Under dry conditions the second aspect of the phytotoxic effect may become important. This depends on the insecticide and solvent chosen and the quantities that are used to formulate fertilizer mixtures. Gojmerac (1956, 1957) showed that some of the organic phosphate insecticides were more phytotoxic than chlorinated hydrocarbon insecticides. This was confirmed with phorate in our tests and it was found that this insecticide inhibited root growth and damaged root tissue. Also Hills *et al.* (1956), Reynolds *et al.* (1957) and Gates (1959) have shown that phorate applied to beet seed at rates in excess of 1.5 pounds per 100 pounds of seed, not only affected seedling emergence but also seedling growth. Fertilizer mixtures containing Diazinon were phytotoxic while those containing Trithion were not.

We may conclude from 2 years' results in Manitoba that 1.25% heptachlor on ammonium phosphate (11-48-0) applied to the seed furrow at 80 pounds per acre, to give 1 pound actual insecticide does not cause serious phytotoxic effects. This method gave efficient root maggot control (Allen *et al.* 1961), but has required a departure from the recommended method of applying fertilizer to sugar beets in Manitoba. Generally, ammonium phosphate (11-48-0) is applied at 65 pounds per acre as a sidedressing at planting time. This practice recognized the fact that furrow application of fertilizer above the rate specified may cause serious phytotoxic effects under condition of low soil moisture (Snyder 1957). However, Dubetz *et al.* (1959) consider that 100 pounds of ammonium phosphate (11-48-0) may be placed with the seed at planting time without causing serious reduction of seedlings.

The phytotoxicity caused by heptachlor-fertilizer applications increased even under ideal soil moisture conditions as the toxicant was increased from 1 to 2 pounds per acre. As phytotoxicity would have been more serious under dry soil conditions, the rate of heptachlor should probably not exceed 1 to 1.5 pounds per acre.

Our results suggest that insecticides or solvents that inhibit root growth strongly or damage root tissue in laboratory tests, may be phytotoxic in fertilizer-mixtures. This was shown for the insecticides Diazinon and phorate and for the solvents heavy aromatic naphtha, and Velsicol AR-60. When such agents are used the phytotoxic effect, under dry soil conditions, will exceed that attributable to fertilizer alone.

Seed treatments of heptachlor, phorate, Trithion or Diazinon at 1 pound actual per 100 pounds of seed, did not significantly decrease seedling emergence from captan-treated seed. However, seed treatment with heptachlor and phorate at this rate reduced emergence from seed that had not been treated with captan. Phorate may depress emergence slightly and there is some evidence that heptachlor in combination with the captan seed treatment enhances the protective effect of captan (Richardson 1960).

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The Uptake and Metabolism of Parathion by Insect Eggs[1]

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ABSTRACT

In an attempt to account for their widely differing susceptibilities to parathion, the uptake and metabolism of P^{32}-parathion vapor by eggs of the large milkweed bug (*Oncopeltus fasciatus* (Dall.)) and peach tree borer (*Sanninoidea exitiosa* (Say)) and the uptake by eggs of the Mexican bean beetle (*Epilachna varivestis* Muls.) and southern armyworm (*Prodenia eridania* (Cram.)) were investigated. In all cases large amounts of parathion were taken up by the chorion and most (50% to 94%) could be washed off with acetone followed by chloroform. Considerable variations were found in ability of the eggs to take up parathion and in penetration of the chorion by the parathion. In the ovicidally susceptible peach tree borer and the nonsusceptible milkweed bug conversion of internal parathion to paraoxon was 30% and 25%, respectively, suggesting that activation and detoxification did not account for the insensitivity of the milkweed bug. In the other three species there was within broad limits a general relationship between internal levels of parathion and ovicidal susceptibility. No single factor could be cited to account for the variations in ovicidal susceptibility of the various species to parathion.

Earlier investigations on ovicidal action of parathion disclosed marked differences in susceptibility of eggs of the peach tree borer, *Sanninoidea exitiosa* (Say), and the large milkweed bug, *Oncopeltus fasciatus* (Dall.) (Smith & Wagenknecht 1956). Susceptibility in the peach tree borer was associated with high levels of cholinesterase inhibition following *in vivo* treatment. By contrast, eggs of the milkweed bug, which are not susceptible at practical concentrations of parathion, showed low levels of cholinesterase inhibition. Substrate specificity studies revealed that the esterases of the two species differed but as esterases from both species were inhibited by *in vitro* treatment with parathion it seemed unlikely that the differential susceptibility was due to dissimilarity in the esterases. A more likely explanation for the insensitivity of the milkweed bug appeared to be that the parathion failed to penetrate the chorion, or if penetration occurred, the parathion failed to become metabolized to the active cholinesterase inhibitor, presumably para-oxon.

In this and the previous studies, the criterion for determining ovicidal susceptibility is the effect of treatment on hatching, *i.e.*, the emergence of the nymph and larva from the chorion. It is known that treatments which do not prevent hatch may later cause mortality in the hatched forms, particularly in the milkweed bug, and this effect is currently being investigated.

The present study was undertaken to determine the rate of uptake, penetration and metabolism of parathion by eggs of several insect species and to consider the relationship of these findings to susceptibility. In addition to the species named, the Mexican bean beetle, *Epilachna varivestis* (Muls.), and southern armyworm, *Prodenia eridania* (Cram.), were also included.

MATERIALS AND METHODS.—*Synthesis.*—P^{32}-parathion was synthesized according to Krueger *et al.* (1960).The product was purified by chromatography on an alumina column, the fraction eluted by benzene being used.

The overall yield was 73%, and the product was identical in infrared spectrum with 99.6% pure parathion. The activity in the liquid counter was 1,197 c.p.m. for 1 μg./ml.

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Treatment.—The eggs used were 1 day old for the milkweed bug, 2 days old for the peach tree borer, 3 days old for the Mexican bean beetle and 3 days old for the southern armyworm. The Mexican bean beetle eggs were left on the leaf segment during exposure and removed just before homogenizing.

Eggs were exposed to parathion vapor in Syracuse watch glasses. A filter paper (Whatman No. 1, 4.25 cm.) impregnated with 0.2 ml. of 2% P^{32}-parathion in acetone was placed in a Syracuse watch glass. Another filter paper treated similarly was attached with a spot of glue to the bottom of another Syracuse watch glass used as a cover. The insect eggs on a sheet of lens paper were introduced into the chamber formed by the two Syracuse watch glasses. Handled in this manner, eggs were exposed to parathion vapor without contact with treated surfaces.

For the main study, which involved eggs of the peach tree borer and milkweed bug, eggs for a given treatment of both species were exposed in the same chamber to eliminate variables in procedure.

The weight of eggs per treatment was 40 mg. except for the peach tree borer, for which (because of the limited supply of eggs) alternating samples of 4 mg. and 40 mg. were used with complete analyses being made only on the 40 mg. samples. Eggs were subjected to variable exposure times with a maximum of 48 hours, zero time being the moment of beginning exposure. After the 48-hour exposure, eggs were removed to untreated Syracuse watch glasses.

Analysis.—For the milkweed bug and peach tree borer, samples were taken during and after exposure at 0.25, 0.75, 1.5, 2.5, and 3.5 days from zero. They were homogenized in 10 ml. of 1% trichloracetic acid, using an iced plastic-and-glass Potter-Elvejhem homogenizer, and counted in a liquid counter. Samples taken 0.5, 1, 2, 3 and 4 days from zero were completely analyzed as follows: (a) Eggs were washed in a small filter funnel with 5 ml. of acetone followed by 5 ml. of chloroform (this treatment had no affect on the appearance of the eggs), the washing being combined and counted as "surface" material. (b) The washed eggs were homogenized in 10 ml. of trichloracetic acid as above and counted, giving "internal" material. (c) 9 ml. of the homogenate was extracted with 15 ml. of chloroform, and the aqueous and chloroform layers separated, filtered and counted giving "chloroform extractables" (this should include parathion, para-oxon and any labeled phospholipids), and "chloroform inextractables" (this should include all hydrolysis products). (d) 10 ml. of the chloroform phase were evaporated to dryness, taken up in 10 ml. hexane, and added to a 6.5×1.3 cm. alumina column (Woelm acid alumina, activity Grade II). Elutions were made with 10 ml. hexane to elute phospholipids, 20 ml. benzene to elute parathion, and 30 ml. chloroform to elute para-oxon (Plapp & Casida 1958). Ten ml. fractions were collected and counted. Appropriate corrections were applied for self-absorption by the solvents used. Egg homogenates had the same self-absorption as distilled water.

For the Mexican bean beetle and southern armyworm, one treatment only (48-hr. exposure) was used, and the eggs were then treated as in (a) and (b) above, without further analysis.

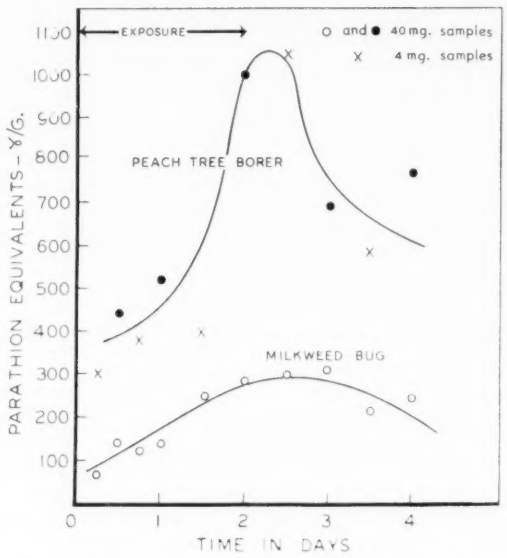

FIG. 1.—Total parathion and metabolites in and on eggs of the peach tree borer and milkweed bug after 2-day exposure to parathion vapor.

Experiments with the alumina columns showed that parathion was always removed with the first 10 ml. of benzene, para-oxon with the second 10 ml. of chloroform. With the procedure described, fractionation was therefore complete. In order to examine recoveries, labeled parathion in chloroform was counted, then put through the column procedure. All appeared in the benzene fraction, the recovery being 105%.

Other experiments demonstrated the sufficiency of the egg-washing procedure ((a) above). For example, the standard washing procedure used on milkweed bug eggs gave in one case washings containing 1,402 c.p.m.; a second such washing yielded 64 c.p.m.; and a third wash with 10 ml. of solvent ether yielded 17 c.p.m.

RESULTS.—Exposures to parathion vapor resulted in the uptake of large quantities of parathion by eggs of both species. As shown in figure 1 the peach tree borer took up about three times as much parathion as the milkweed bug. Results for the peach tree borer differ depending upon whether the samples were of 4 mg. or 40 mg., the latter giving a somewhat higher yield per mg. We are at a loss to account for this. It is probably not caused by the fact that one set was homogenized directly while the other was washed first, since with the milkweed bug eggs there was fair agreement where two kinds of treatments were involved, both with 40 mg. samples. It is interesting to note that parathion was lost from the eggs when exposure to it was terminated, this loss being from the external surface of the egg as shown in figure 2.

Most of the parathion taken up was external and could be removed by washing with organic solvents. For the peach tree borer, an average of 79% was external compared with 92% for the milkweed bug. It was shown that

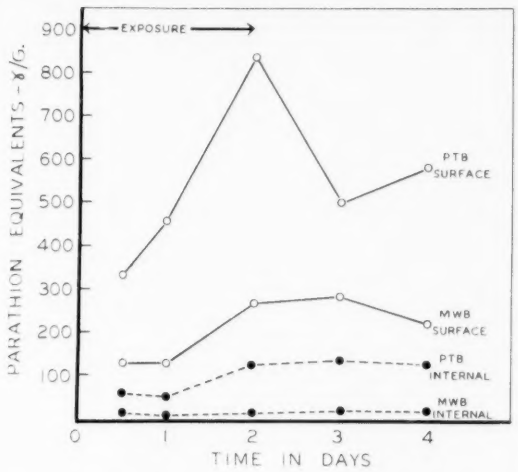

FIG. 2.—Surface parathion and internal parathion-plus-para-oxon in eggs of the peach tree borer and milkweed bug after exposure to parathion vapor.

the external material was mainly unaltered parathion; 99% in the case of the milkweed bug and 92% in the case of the peach tree borer.

The internal levels were further fractionated and the level of chloroform-extractables (mainly parathion plus para-oxon) found to be about eight times higher in the peach tree borer than the milkweed bug. Finally, the actual levels of parathion and para-oxon inside the eggs are shown in tables 1 and 2. The maximum level of para-oxon in the peach tree borer was 47 mg./kg., in the milkweed bug 5 mg./kg. These levels represent conversion of 30% and 25%, respectively, of internal parathion to para-oxon. The level of para-oxon declines slightly after termination of exposure, suggesting degradation in both species.

Experiments with milkweed bug eggs (fig. 3) showed that there was a direct relationship between the concentration of parathion used, the amount taken up outside, and the level reached inside. This being the case it is surprising that much higher concentrations than those shown here, *e.g.* 99%, do not result in internal levels which prevent hatch. (See table 3.)

The uptake of parathion by two other species is given in table 3, together with data on size and relative suscepti-

Table 1.—The uptake, penetration and metabolism of P^{32}-parathion by eggs of the peach tree borer.[a]

Sampling Time (days)	Total	Surface	Internal	Internal		Internal $CHCl_3$		
				H_2O	$CHCl_3$	Phospholipid	Parathion	Para-oxon
0.25	287							
0.5	444	331	113	19	60	3	62	23
0.75	366							
1	525	456	69	19	50	2	41	23
1.5	375							
2	998	839	159	17	125	2	70	47
2.5	1,042							
3	689	494	195	30	131	2	82	41
3.5	558							
4	767	585	182	14	128	5	108	34

[a] Values calculated as μg. gm. of parathion. All the data are direct measurements, except for "total" parathion, which is where possible the sum of the "surface" and "internal." Eggs exposed for 2 days.

Table 2.—The uptake, penetration and metabolism of P^{32}-parathion by eggs of the milkweed bug.[a]

Sampling Time (days)	Total	Surface	Internal	Internal		Internal $CHCl_3$		
				H_2O	$CHCl_3$	Phospholipid	Parathion	Para-oxon
0.25	66							
0.5	144	127	17	0.6	10	5	15	0
0.75	123							
1	137	129	8	0.6	7	1	7	0
1.5	246							
2	286	266	20	1	15	3	12	5
2.5	296							
3	308	284	24	1	17	3	14	2
3.5	210							
4	245	219	26	4	17	1	19	3

[a] Values calculated as μg. gm. of parathion.

Table 3.—Relationship between parathion uptake in 2 days, size of eggs and relative ovicidal susceptibility.[a]

	Peach Tree Borer	Milkweed Bug	Mexican Bean Beetle	Southern Armyworm
Total uptake (μg. parathion equivalents per gm.)	998	286	300	116
Internal level (μg. parathion equivalents per gm.)	159	20	150	36
Internal parathion as per cent of total	16	7	50	31
EC_{50}: Concentration of parathion (%) to inhibit emergence 50%	0.008	>99.9	.015	.007
Size of egg:				
Volume, mm^3.	0.04	0.26	0.24	0.04
Surface, mm^2.	0.62	2.12	2.05	0.62
Weight, mg.	0.05	0.26	0.26	0.05
Surface per unit weight (mm^2. $mg.^{-1}$)	12.4	8.2	7.9	12.4

[a] The uptake results were based on the standard concentration of parathion, *i.e.*, 0.2 ml. of 2% per filter paper. Figures in lines 1–3 are for parathion-plus-metabolites. The concentration in line 4 represents the per cent parathion on filter paper to produce 50% ovicidal effectiveness from 48-hour exposure.

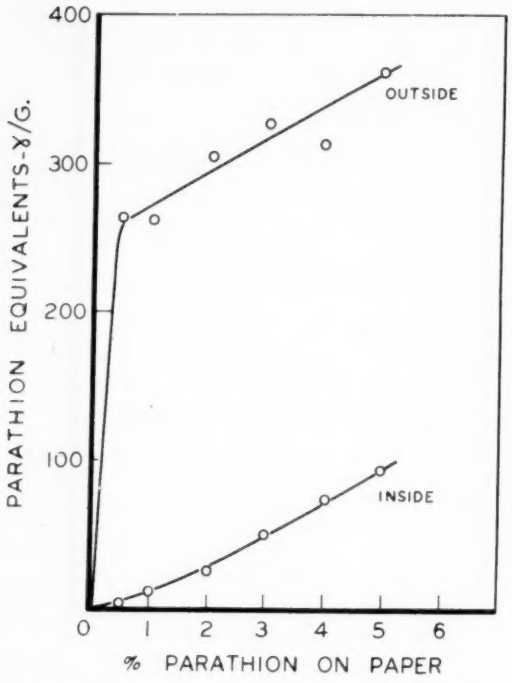

FIG. 3.—Effect of parathion concentration applied to filter papers on the internal and external levels of parathion-plus-metabolites in milkweed bug eggs exposed for 2 days.

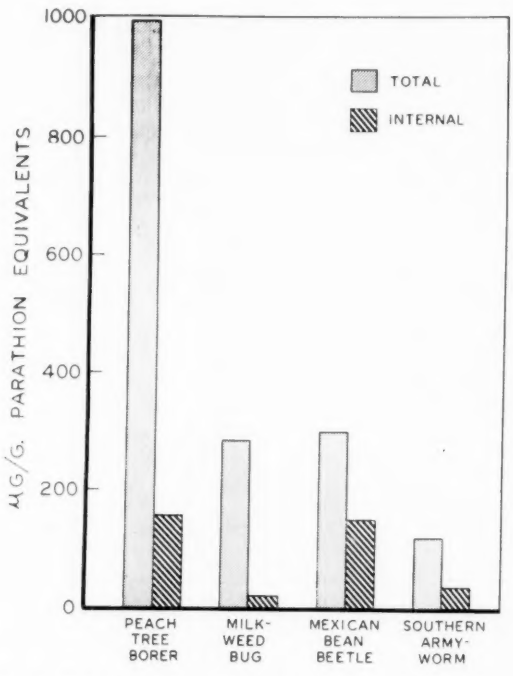

FIG. 4.—Total uptakes and internal levels of parathion-plus-metabolites in eggs of four species following 2 days' exposure.

bility. The relationships between external and internal levels for the four species are also presented graphically in figure 4. While surface area of eggs is likely a factor influencing total uptake, this does not explain the differences shown: the peach tree borer and southern armyworm are of equal size with a ninefold difference in total uptake. A comparison between volume and total uptake shows essentially the same relationship as applies to surface area.

The levels of para-oxon in the Mexican bean beetle and southern armyworm were not determined.

DISCUSSION.—Differential susceptibility to parathion treatment could be caused by differences in one or more of the following factors:

1. Rate of uptake
2. Rate of penetration of the chorion
3. Rate of conversion to active inhibitor (presumably para-oxon)
4. Detoxification

5. Failure of the toxicant to reach the target (presumably cholinesterase)
6. Sensitivity of the target to inhibition.

The present investigation provides some evidence on the first three factors. With respect to the first factor, that of uptake (table 3), all four species took up parathion at high rates, the maximum variation among the species being ninefold. This variation was not correlated with variations in the surface area per unit weight (maximum variation occurred between species whose eggs were identical in weight and size) and was therefore probably caused by differences in the nature of the chorion. There was no correlation between total uptake and susceptibility to poisoning.

With respect to penetration there was no correlation between total uptake and "internal level as per cent of total." For example, as figure 4 shows, the Mexican bean beetle achieved an internal level equivalent to that of the peach tree borer in spite of a total uptake of only a quarter as much, indicating differential penetration.

In only two cases is there evidence on conversion to para-oxon. Here conversion was of the same order being 25% and 30%, respectively, in the milkweed bug and peach tree borer. If these results are typical most of the internal material was parathion and this was also true for the external material as mentioned above.

From the data of tables 1 and 2, there appears to be clear correlation between internal para-oxon level and toxicity, for very high levels of para-oxon (*e.g.*, 47 μg./gm.) were achieved in the susceptible peach tree borer and low levels (maximum 5 μg./gm.) in the nonsusceptible milkweed bug. Unfortunately comparable data are not available for the other species. These data appear at first to account satisfactorily for the selectivity and one is inclined to argue that the low internal para-oxon of the milkweed bug was due to the correspondingly low internal parathion level, which in turn was due to differences in total uptake. If this were so, the selectivity would be caused by differences in uptake properties of the chorion. However, in view of the fact mentioned above that extremely high parathion doses (which lead to high internal levels of parathion-plus-metabolites, figure 3) are not ovicidally effective, it is probable that some additional factor must be evoked to explain the insensitivity of this species. The obvious possibilities are (a) low internal para-oxon level in spite of high internal parathion-plus-metabolites levels, (b) failure of para-oxon to reach its target, (c) insensitivity of the target to para-oxon. However, with regard to (a), the percentage of internal parathion-plus-metabolites present as para-oxon was so similar in the two species (30% and 25%) that it seems improbable that either degradation or activation could be much different in these species.

By comparison with the other three species, the milkweed bug poses an anomaly, apparently having no threshold of ovicidal susceptibility to the treatments employed. This insensitivity is likely due to failure of the para-oxon to reach its target (esterases?) or to insensitivity of the target to para-oxon.

In the other three species, while close correlations between internal level and susceptibility are lacking, the differences are much less. Susceptibility varies by twofold and "internal level" by fourfold. The southern army worm is relatively more sensitive than would be expected based on its internal parathion level; possibly the activating system is more effective or the esterases more sensitive.

From the evidence cited it appears that no single factor accounts for variations in susceptibility and it will be necessary to evaluate all of the above possibilities in order to establish the reasons for selective toxicity of parathion to the eggs of various species.

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SCIENTIFIC NOTES

Emergence of Overwintered Pink Bollworm in Arizona[1]

GEORGE P. WENE,[2] L. W. SHEETS,[3] and H. E. WOODRUFF[4]

In 1958 the pink bollworm, *Pectinophora gossypiella* (Saunders), was found in Maricopa County in central Arizona for the first time in 10 years, although relatively light infestations have been continuously present in Graham County in eastern Arizona for a number of years. This finding in Maricopa County resulted in an intensified eradication program involving the destruction and plowing under of crop remnants from the previous season and the delayed planting of new crops. In an attempt to obtain information on the survival and emergence of the pink bollworm under Arizona conditions on which to base future eradication efforts, two tests were conducted, one in 1958 in eastern Arizona near Safford, and one in 1959 in central Arizona near Phoenix. In each test the emergence cages were similar to those described by Shiller (1946).

In January 1958, 30 bolls from a field heavily infested with pink bollworms were placed in each of 10 standard emergence cages. In one cage bolls were placed on the soil surface. In the other cages bolls were buried at several depths under the soil as follows: 3 cages—1 inch, 1 cage—2 inches, 1 cage—3 inches, and 4 cages—4 inches. The soil in the cage with bolls buried 2 inches and in one of the cages with bolls buried 4 inches was irrigated with 2 inches of water shortly after the bolls were buried. The soil in all cages received natural rainfall, 5.49 inches for the first 6 months. The average normal rainfall at Safford for this period is 2.72 inches. In the spring the cages were examined daily and the number of emerging moths recorded. Pink bollworm moths started emerging at the end of March and continued until June 23. The total number of 15 moths emerging was too small to determine a peak of emergence. However, before June 1 twelve of these moths, or 80%, had emerged "suicidally," or before cotton squares were available as food and shelter for larvae hatching from eggs laid by these moths. In this area cotton-planting was prohibited until April 10 and fruiting, as indicated by the appearance of cotton squares, did not occur until June 1 or later in most fields. Data from the small emergence indicated that as many moths emerged from bolls buried 4 inches as from bolls buried 1 inch.

In January 1959, 175 cotton bolls taken from heavily infested fields were placed in each of 10 emergence cages. In two cages the bolls were placed on the soil surfaces. In the other cages bolls were buried at several depths under the soil surface as follows: 4 cages—2 inches, 2 cages—4 inches, and 2 cages—6 inches. All cages were irrigated with 3 inches of water on March 6 and again on April 27. The soil in all cages received natural rainfall, 0.91 inches for the first 6 months. The average normal rainfall at Phoenix for this period is 2.84 inches.

Weekly records of moth emergence from these cages are summarized in table 1. The first pink bollworm moth emerged on March 19 and the last on July 13. Heaviest emergence occurred between April 12 and May 30. In this area the planting date was delayed by regulatory order until April 1 for short staple cotton and cotton squares did not appear until about May 25. At that time 77% of the pink bollworm moths had emerged. The data from these tests indicate that delayed planting of cotton may be one method of controlling or reducing pink bollworm infestations.

More moths emerged from buried bolls than from those on the soil surface. The average number per cage in the 1959 experiment was as follows: 0 inches—9.5; 2 inches—17.0; 4 inches—19.5; and 6 inches—13.5. These results are contrary to those of Fife *et al.* (1957) based on tests at Waco, Texas, in an area with an average annual rainfall of more than 26 inches. However, the data support observations by Olendorf (1926) made in an arid region of Mexico having an average annual rainfall of about 8 inches which is similar to that of Phoenix, Arizona.

Table 1.—Seasonal emergence of pink bollworm adults at Phoenix, Arizona, 1959.

TIME OF EMERGENCE	TOTAL MOTHS FROM 10 CAGES
March 15–21	3
March 22–28	0
March 29–April 4	1
April 5–11	0
April 12–18	12
April 19–25	20
April 26–May 2	24
May 3–9	18
May 10–16	21
May 17–23	19
May 24–30	14
May 31–June 6	7
June 7–13	4
June 14–20	9
June 21–27	0
June 28–July 4	0
July 12–18	1

The observations reported indicate that the relatively high overwintering survival of pink bollworms buried in Arizona soil may be caused by the low rainfall usually experienced in an arid area.

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[1] Journal Paper No. 596 University of Arizona Agricultural Experiment Station. Accepted for publication July 14, 1960.

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[3] Agricultural Research Service, U. S. Department of Agriculture.
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Control of the Beet Armyworm on Cotton in Arizona[1]

GEORGE P. WENE[2] and L. W. SHEETS[3]

During July 1959, heavy populations of the beet armyworm, *Spodoptera exigua* (Hbn.), developed on cotton in the Hidden and Rainbow valley areas of south central Arizona. As many as 60 larvae were found on some plants. In these heavy infestations, the larvae consumed a major portion of the foliage and also fed on the squares and immature bolls in a manner similar to that of the bollworm (*Heliothis zea* (Boddie)). In one field, where the beet armyworm had destroyed most of the leaves, squares and immature bolls, half-grown larvae also bored into the stems about 3 to 6 inches from the terminals, causing the stems to die back to a short distance below the entrance holes. In light in-

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[2] University of Arizona.
[3] Agricultural Research Service, U. S. Department of Agriculture.

festations, having an average of one, or less than one, beet armyworm larvae per plant, the feeding is usually confined to the cotton leaves; it is only when large portions of a majority of the leaves are destroyed by heavy infestations of beet armyworms that the larvae move to the squares and mature bolls for food. Ordinarily, beet armyworm egg masses are deposited on the lower surfaces of cotton leaves, but in these fields, during the period of heaviest infestations, egg masses were also readily observed on the upper surfaces.

The heaviest infestations were found in fields where the cotton plants were the shortest. A survey made near Buckeye, Arizona during July showed that severest infestations were found in fields where the plants were short and slow-growing; fields with rank or rapidly growing plants had only light populations, which were considered noninjurious. The initial severe infestations were adjacent to desert areas. Many of these severely infested fields had received eight applications of DDT dust (a total of 23 pounds of technical DDT per acre) made at 6-day intervals, during an area-wide program for the attempted eradication of the pink bollworm. (*Pectinophora gossypiella* (Saund.))

DDT, either in a dust or a spray, was recommended for the control of the beet armyworm in Arizona prior to 1960 (Roney & Wene 1959). This was based on the work of Stevenson & Kauffman (1948) which showed that good control of this insect was obtained with one application of a dust containing 10% DDT applied at 12 to 20 pounds per acre. Resistance of the beet armyworm in Arizona to DDT was reported by Wene (1959) and was confirmed by the build up of beet armyworm populations in fields treated with DDT during 1959 in the pink bollworm eradication program. Beet armyworm populations also built up in a field which was dusted eight times at 6-day intervals with a mixture of 15% toxaphene, 10% DDT, plus 30% sulfur. One field in the pink bollworm eradication program area was sprayed 16 times with a mixture of toxaphene-DDT (3 and 1.67 pounds, respectively, per application), after which beet armyworms were still collected in the samples collected. Due to the failure of recommended insecticides to control heavy beet armyworm infestations, other insecticides were tested in a series of seven experiments conducted during 1959.

All experiments were conducted in severely infested fields. All treatments were applied by airplane to nonreplicated plots of from 4 to 5 acres in area. One, two, or three days after treatment, 10 or 20 plants were selected at random in each plot and the beet armyworm population determined by shaking the cotton plant so that the larvae dropped on a heavy white cloth. Because of the damage being done, growers would permit check plots to remain untreated only for periods of varying from 24 to 48 hours. Each of the seven experiments was conducted in a different field. Growers were demanding the recommendation of an insecticide that would control beet armyworms in a period of 24 to 72 hours. Because of the severity of the infestations, slower killing insecticides were of little value as far as the growers were concerned. The insecticides applied and the results obtained are shown in table 1.

The data in table 1 show that, as a spray, Dipterex® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) at 1.5 pounds per acre gave effective control. DDT and toxaphene were added to the Dipterex spray in some of the experiments in order to prevent a build up of bollworms. The data show that a dust formulation of Dipterex was less effective than the sprays. A spray mixture consisting of 0.5 pound Dibrom® (1,2-dibromo-2, 2-dichlorethyl dimethyl phosphate) plus 0.4 pound endrin and one containing 1 pound of Dibrom plus 0.2 pound of endrin per acre were slightly less effective. Good control was also obtained with a spray mixture consisting of 0.9 pound of Dilan® (1 part of 1,1-bis(*p*-chlorophenyl)-2-nitropropane plus 2 parts of 1,1-bis (*p*-chlorophenyl)-2 nitrobutane) plus 0.3 pound of endrin per acre. Methyl parathion applied as a spray at 2 pounds per acre and a spray mixture consisting of 0.5 pound of methyl parathion plus 0.4 pound of endrin per acre gave poor control.

Table 1.—Control of the beet armyworm with airplane applications of various insecticides. 1959.

Treatments and Dosage/Acre	Days After Treatment	Average No. Larvae per Plant	Control (%)
Experiment 1			
30 lb. 5% Dipterex+10% DDT, dust	1	9.2	64
Untreated	1	25.6	—
Experiment 2			
30 lb. 5% Dipterex+10% DDT, dust	1	4.0	64
Untreated	1	11.2	—
Experiment 3			
1.5 lb. Dipterex+2 lb. DDT, spray	3	0.1	98
Untreated	3	4.0	—
Experiment 4			
1.5 lb. Dipterex, spray	2	0.2	80
2.0 lb. Methyl parathion, spray	2	0.5	50
Untreated	2	1.0	—
Experiment 5			
1.5 lb. Dipterex, spray	3	1.0	91
0.9 lb. Dilan+0.3 lb. endrin, spray	3	1.1	91
0.5 lb. Methyl parathion+0.4 lb. endrin, spray	3	4.6	57
Untreated	3	10.8	—
Experiment 6			
1.5 lb. Dipterex+1.0 lb. DDT, spray	2	2.0	92
0.5 lb. Dibrom+0.4 lb. endrin, spray	2	5.6	79
Untreated	2	26.5	—
Experiment 7			
1.5 lb. Dipterex+3 lb. toxaphene, spray	2	1.5	85
1.0 lb. Dibrom+0.2 lb. endrin, spray	2	2.2	78
0.8 lb. endrin, spray	2	7.9	22
30 lb. 5% Dipterex, dust	2	2.3	73
30 lb. 2.5% Guthion, dust	2	7.8	23
30 lb. 7.5% Sevin, dust	2	10.1	0
Untreated	2	10.1	—

Endrin, applied as a spray at 0.8 pound per acre, gave very little control. Dust formulations of Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazine-3-(4*H*)-ylmethyl) phosphorodithioate) and Sevin® (1-naphthyl methylcarbamate) also failed to control beet armyworms.

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Scabbing of Rutabagas After Applications of Heptachlor, Parathion, and Lime to Mineral Soils of Various Acidities[1]

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From 1955 to 1959, observations in fields throughout Prince Edward Island showed that up to 40% of rutabagas grown in soil that had received preplanting treatments of heptachlor against root maggots were damaged by scab on the portions of the roots below ground level. This is a report on an experiment in 1959 on whether insecticides or lime, or both, applied to soils of various levels of acidity influenced the degree of scab infection on rutabagas.

[1] Contribution No. 62, Research Laboratory, Research Branch, Canada Department of Agriculture, Charlottetown, Prince Edward Island, Canada. Accepted for publication August 1, 1960.

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Table 1.—Percentages of rutabagas infected with scab after application of heptachlor or parathion to soils of various acidities, and to soils to which various amounts of limestone were applied at seeding.

	Percentage of Rutabagas Infected					
	No Lime Applied; Soil pH at Harvest					
Insecticide[a]	5.00	5.28	5.41	5.56	5.68	6.39
Untreated	0	0	0	0	0	12
Heptachlor	0	0	7	10	32	20
Parathion	0	0	5	15	30	18
	Pounds/Acre Lime at Seeding; Soil pH at Harvest					
	0	500	1,000	2,000	3,000	4,000
	5.00	5.50	5.61	5.78	6.23	6.68
Untreated	0	0	0	0	8	6
Heptachlor	0	0	13	35	65	65
Parathion	0	0	15	30	80	70

[a] Applied at 5 lb./acre with a subsurface applicator to give 5-inch bands 1½ inches below the seed in planting ridges.

Methods.—The experiment was conducted on an area divided into 6 sections, with 4-foot buffer strips between sections. The sections had been treated in a rotation at 5-year intervals, beginning in 1937, with ground limestone at 0 to 4000 pounds per acre, the last application having been made in 1956. In the present experiment, half of each section was again treated at seeding with lime at the same rates as had been used in the rotation plan. The pH of the soil in each subsection, determined from samples taken at harvest, is given in table 1. The lime was broadcast and raked into the upper 3 inches of soil.

The insecticides, granules of 5% heptachlor and parathion, were applied with a machine that sowed the rutabaga seed and applied the precise amount of insecticide in the ridged row; rates and methods of application are given in table 1. The insecticide treatments and one untreated plot were replicated three times in single-row, 20-foot plots in each subsection. All treatments were randomized.

Laurentian rutabagas were planted on June 15 and final records were taken on October 25. All of the plants in the plots were examined for scab.

Results.—Where no insecticide was applied, there was no scab injury on the rutabagas grown in the more acid soils (below pH 5.78) and only slight infection on those in the less acid soils, whether or not lime was applied at seeding (table 1). At pH values from 5.4 to 6.4 both heptachlor and parathion caused a marked increase in the percentage of infection, and in the subsections where lime was applied together with insecticide injury was moderate to very severe. In the subsections with pH 6.2 to 6.68, some of the insecticide and lime-treated plots had 100% infection, the portions of the rutabagas below ground level being almost completely covered with lesions.

There was no significant difference in yield between plots or subsections, although the soils with pH values from 5.5 to 6.0 produced the best type of rutabagas. At higher pH levels, the plants were excessively rooty.

Work now in progress indicates that the pathogen causing the scab on rutabagas is similar to common potato scab, *Streptomyces scabies*. Isolates from scab lesions on both potatoes and rutabagas are morphologically similar and have been found equally pathogenic on Green Mountain potato tubers.

Discussion.—The insecticides evidently promoted development of the pathogenic organism, either directly or by breaking down a protective mechanism within the plant. Also, although lime did not in itself cause any noticeable effect, there was evidently a synergistic effect on development of scab infections when lime was used in combination with insecticide, although the mechanism of such action is not yet known.

Further work on the scab organism and other phases of the problem is being continued. The results reported herein suggest that lime should not be applied to mineral soils where soil treatments of either chlorinated hydrocarbon or organic phosphate insecticides are used against insects attacking rutabagas.

Influence of Water and Previous Food on the Longevity of Unfed Larvae of *Coleomegilla maculata lengi*[1]

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Ability to survive at low density of prey increases the effectiveness of entomophagous predators. When food is scarce the quality of the available food may increase survival by prolonging the life of the predator and thereby increase its chance of locating food. This is perhaps most important for predators that have relatively weak powers of perception, such as those studied by Fleschner (1950) that were unable to perceive the prey until a physical contact was made. Banks (1956) showed that newly hatched larvae of the coccinellid, *Adalia bipunctata* (L.), that ate one egg of their own species lived twice as long as unfed larvae, but that water extended the life of unfed larvae by only a few hours. The effects of water, a sugar solution, and previously eaten food on the longevity of unfed larvae of *Coleomegilla maculata lengi* Timb. (Coleoptera: Coccinellidae) are discussed below.

Materials and Methods.—*C. maculata* adults were kept in petri dishes and larvae in cells as Smith (1960) described for rearing coccinellids on dry food. The larvae used were the progeny of 10 females, three of which were fed the dead nymphs and adults of the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae). These aphid stages were dried at approximately 20° C. and reduced to a fine powder. The food given to the other adults was mainly composed of desiccated liver (Difco Co.). To reduce variation, larvae from the same egg mass were distributed to receive the following treatments: shortly after eclosion one-third of the first-instar larvae of an egg mass was placed individually in cells and given distilled water but no food; one-third was treated similarly except that no water was provided; and one-third was treated similarly except that a 5% solution of sucrose was provided. This procedure was repeated with larvae from other egg masses until there were at least 15 larvae in each group. The larvae were inspected frequently until death and the time lived by each was recorded. In other tests, half the first-instar larvae were reared on powdered aphids and half were reared on a dry powdered food composed mainly of brewers' yeast (Mead Johnson and Co.). Both groups were given water separately. Immediately after the first moult the second-instar larvae of both groups were divided; half of each was given water but no food, and half was given neither water nor food. Enough larvae were started so that the time lived from the first moult to death was recorded for at least seven individuals in each of the four groups. Larvae were similarly reared to the second and third moults for com-

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Table 1.—Mean longevity and difference in the means for unfed larvae of *C. maculata* with water and without water and reared on *R. maidis* and on yeast.[a]

Treatment	Second Instar		Third Instar		Fourth Instar	
	Days Lived	Difference Between Foods	Days Lived	Difference Between Foods	Days Lived	Difference Between Foods
Water	4.2 (3.6)	0.6	4.2 (3.0)	1.2	5.2 (4.7)	0.5[b]
No water	3.4 (2.7)	0.7	3.1 (2.0)	1.1	4.2 (3.4)	0.8
Difference between treatments	0.8 (0.9)		1.1 (1.0)		1.0 (1.3)	

[a] Results for yeast given in parenthesis.
[b] Significant at 5% level by F test. All other differences significant at 1% level.

parisons with third- and fourth-instar larvae. The work was done in the laboratory under controlled conditions of about 22° C. and 65% relative humiditiy.

RESULTS AND DISCUSSION.—The first-instar larvae given the sucrose solution lived 15.1±5.5 days, but did not moult. The time lived by unfed first-instar larvae was considerably shorter, but those given water lived longer than those without water. The mean number of days lived on water was 3.7 and without water was 3.2: the difference was significant by the F test ($P<.05$). When reared on *R. maidis*, each of the three larval instars was shorter than when reared on yeast. However, the differences between durations were significant ($P>.05$) only for the second instar. Unfed second-, third-, and fourth-instar larvae of *C. maculata* lived longer ($P<.01$) when given water and when reared on *R. maidis* than when reared on yeast (table 1).

Water increased the length of life of unfed first-instar larvae by about 16%. The need for water to replace that lost by respiration and excretion is reflected by this increase. The magnitude of the increase is probably in part attributable to small differences in the relative humidities of the rearing chambers used. The addition of 5% sucrose to the water further increased the life of the first-instar larvae by more than 300%. The times lived by the other instars with and without water indicate that the older larvae are better able to survive starvation, as Jacobson & Blakeley (1960) found also for *Agrotis orthogonia* Morr. (Lepidoptera: Noctuidae). Water encountered in the field by polyphagous species, such as *C. maculata*, probably contains various substances that may increase survival to a greater degree than the sucrose added to the water used here.

The author has found that *C. maculata* can be reared from first-instar larva to adult on the powdered stages of *R. maidis*, and also on yeast with relatively small mortality the details of which will be published later. It is not surprising, therefore, that no important differences were found between the developmental rates on these two foods. However, water had a greater influence on the length of life of unfed larvae reared on yeast than on those reared on *R. maidis*. The percentage increases in times lived by the various instars reared on *R. maidis* and (in parentheses) yeast were: second instar, 23 (33); third instar, 35 (50); and fourth instar, 24 (38). The larvae reared on aphids may have contained more water than those reared on yeast and therefore were better able to withstand desiccation. This would explain why the increases in the times lived by larvae reared on aphids over those reared on yeast are greater for individuals not given water than for those given water. The percentage increases in time lived by the various instars without food but with water and (in parentheses) without water were: second instar, 17 (26); third instar, 40 (55); and fourth instar, 11 (24).

The literature contains little detailed information on the influence of food quality on the rate of development of coccinellids. A possible reason for this is the difficulty of standardizing animal foods such as aphids and scales. Putman (1957) found that the larvae of the two coccinellids *Hippodamia convergens* Guer. and *Adalia bipunctata* (L.) required nearly twice the time for development when fed mites as when fed aphids. He found a smaller but significant difference for *C. maculata*, but suggested that the difference here was caused by the quantity of food rather than quality as this predator spent more time searching for mites than for aphids. It is known that many species of coccinellids eat foods other than the usual prey, particularly when the latter are scarce. Watson & Thompson (1933) showed that some fed at the nectar glands of plants and on sap, pollen, fungi, and honeydew. Some of these substances are probably nutritionally inadequate for normal growth but can supply sufficient energy to enable the predator to continue searching for foods of better quality. The sucrose solution used in the present studies is an example of a subsistence type food with potential survival value. The control value of many aphid-feeding coccinellids is often limited by their inability to find sufficient food at low prey density to bring them to maturity. The present study shows that water and food quality affect the length of life of larvae of *C. maculata* in the laboratory. In the field these influences may be of importance in determining the population density of a predatory coccinellid.

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Biological Notes on Five Recently Imported Parasites of the Egyptian Alfalfa Weevil, *Hypera brunneipennis*[1]

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From January through May 1960, 32 shipments of parasitized eggs, larvae, or prepupae of *Hypera* species were received from Iran. The following species of parasites currently being studied are: *Patasson* (=*Anaphoidea*) sp., a mymarid egg parasite; *Bathyplectes curculionis* (Thoms.) and *B. corvina* Thoms., both ichneumonids, which oviposit in young weevil larvae; and the pteromalids *Dibrachoides druso* (Walker) (=*dynastes* Foerster of authors), which attacks mature larvae, particularly those within cocoons, and *Habrocytus* sp., which attacks prepupae and young pupae within their cocoons. Determinations of these species were provided by C. F. W. Muesebeck and L. Walkley, U. S. Department of Agriculture, Washington, D. C.

Reported here is a continuation of attempts to achieve biological control of *Hypera brunneipennis* (Boh.). This is a follow-up of recommendations made by van den Bosch & Dietrick (1959), who pointed out that *H. brunneipennis* is partially immune to attack by the larval parasite *Bathyplectes curculionis* which was successfully introduced from Italy against *H. postica* (Gyll.) into Utah (Chamberlain 1926). This ichneumonid wasp was introduced into California where it has been credited with good biological control of *H. postica* (Michelbacher 1943).

In 1911 the species known now as *Dibrachoides druso* and *Bathyplectes curculionis* were introduced into Utah from Italy in an attempt to control the alfalfa weevil, *Hypera postica*. The current reimportation of these (presumably) same species was considered justifiable because they were from a hot, dry area and, more important, from a different host. *Patasson* sp., *Habrocytus* sp., and *B. corvina* are new introductions against *Hypera brunneipennis*.

In order to understand some of the problems encountered in the laboratory propagation of *Hypera brunneipennis* and colonization of its parasites in the field, a summary statement regarding the biology of the weevil is given.

Hypera brunneipennis is found throughout most of southern California on alfalfa, bur clover, etc. It is most severe in the hot Imperial Valley where it causes economic damage on first and second cuttings of alfalfa hay. In this area adult weevils in reproductive diapause usually seek shelter beneath the loose bark of trees, particularly *Eucalyptus* spp. They emerge in December and January, re-enter the hay fields, and begin feeding and mating. Oviposition in the pithy, hollow stems of alfalfa begins in February and may extend into April. Almost all of the adults which develop from this spring generation feed heavily, and leave the fields, without ovipositing, by May or early June and enter summer diapause. By this time all of the old ovipositing adults from the preceding year have died. This univoltine cycle imposes problems of synchronizing generations of parasites with that of the weevil and virtually restricts attempts at field colonization to March and April, the only period when eggs, larvae, and pupae of the host occur simultaneously.

Numerous laboratory and field experiments have been tried in order to effect an early breaking of diapause in the adult weevil as well as to prevent the adults from entering diapause. At times we have been successful without really knowing how it was done. On the other hand, it was determined that approximately 1% to 2% of the new spring diapausing population does not enter diapause, but rather will commence laying eggs after a prolonged feeding period. By rearing large numbers of weevils in the insectary (5,000 or more per month), together with collecting large numbers of field adults, we have been able to produce a steady supply of eggs from which the various stages of the weevil and the several species of parasites have been maintained.

Complications of rearing weevils and parasites included the predatory feeding of the mite *Glycyphagus domesticus* (De Geer) and the flower thrips *Frankliniella tritici* (Fitch) on the weevil eggs deposited inside the alfalfa stems. Clean culture as well as use of the predatory mite *Blattisocius tenuivorus* Oudemans, which fed voraciously on *Glycyphagus*, soon resulted in satisfactory production.

Preliminary biological notes for each species of parasite are given at this time in order to aid in future field bio-ecology studies which will commence in the late fall of 1960.

Patasson (=*Anaphoidea*) *sp.*—This mymarid develops as a solitary endoparasite on eggs of *Hypera brunneipennis*. The life cycle, *i.e.*, egg to adult, at 80° F. is 11 days, at 70° F. 17 days. Oviposition occurs in the green alfalfa stem, through unplugged punctures made by ovipositing weevils, or directly into exposed weevil eggs. Weevil eggs exposed to direct attack by splitting alfalfa stems lengthwise 12 to 24 hours after *Hypera* egg deposition are suitable for laboratory propagation of the species. By this method, 100% parasitization of weevil eggs and emergence of adult *Patasson* are commonly achieved. In a natural situation a rather high percentage of weevil eggs escape parasitization and many *Patasson* do not find their way out of the stem. Normally *Patasson* emergence lags several days behind eclosion of first-instar weevil larvae, and exit from the stem for both newly hatched *Hypera* larvae and adult *Patasson* is via the oviposition puncture made by the adult weevil. The weevil eggs possess a rather tough chorion and good development of both weevil embryos and *Patasson* occurs at 70° and 80° F., with constant relative humidity of 50%. *Patasson* shows no tendency toward diapause when reared in the laboratory and reproduces generation after generation on fresh eggs of *Hypera*. This is rather curious in light of the characteristic summer diapause of the adult weevil. The following explanations are suggested: (1) in the field the species may find suitable alternate hosts, *i.e.*, eggs of other species of weevils in which to oviposit; (2) adults may live through the summer as diapausing adults; or (3) in certain areas developing immatures of *Patasson* may enter diapause in the egg of the host and pass the summer in the dry alfalfa stubble.

By way of experimentation, fresh eggs of the coccinellids *Chilocorus* and *Hippodamia*, and the moths *Ephestia* and *Sitotroga*, were offered *Patasson* but no oviposition occurred. In laboratory propagation units adults lived 7 to 12 days and displayed strong attraction to water when made available from cotton plugs in vials containing bouquets of alfalfa. No hyperparasites were reared from the Iranian shipments of *Patasson*.

The *Patasson* sp. is distinct from other mymarids introduced against several *Hypera* spp. with the possible exception of a species determined as *Anaphoidea* sp. introduced but not established in California in 1934.

Bathyplectes curculionis—This species was received early in the season, and it readily attacked 2 to 4 mm. *Hypera* larvae. The tests were suspended because all of the progeny entered diapause which has thus far defied attempts at termination. On hand are several hundred F_1 cocoons, each of which contains a mature *Bathyplectes* larvae, and it is hoped that adults will issue in October or November. There is a strong indication that multiple ovipositions are required for development to occur on *H. brunneipennis*.

Issuing along with *Bathyplectes curculionis* was an equal-sized ichneumonid, *Mesochorus nigripes* Ratz., a secondary parasite. This is a striking case of size and coloration resemblance. The

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only superficial difference is that *Bathyplectes* has an upwardly curved ovipositor, while that of *Mesochorus* is straight, and the males of *Mesochorus* possess a prominent pair of exposed caudal cerci, while those of male *Bathyplectes* are smaller.

Bathyplectes corvina.—Cocoons of *Bathyplectes corvina* first appeared in the May shipments by van den Bosch from Teheran, Iran. This species is nicknamed the "Jumping *Bathyplectes*." All cocooned larvae were apparently in diapause, yet they readily jumped when disturbed. Within the cocoon of *Hypera* this behavior is not spectacular, but when *B. corvina* cocoons were removed from those of their hosts, they jumped vertically 1½ to 2 inches. The larval musculature which makes this possible has not been thoroughly studied, but the gross aspects as seen by means of transmitted light are of interest.

The larva braces itself against each end of the oblong cocoon with the midsection of the body curved down toward the substrate. Jumping occurs when the body is snapped upward against the opposite side of the cocoon. This behavior may continue for several minutes after the initial stimulus, such as merely rolling or touching a cocoon. The purpose of such activity is not fully understood. It seems probable that it is a defense mechanism against the attack of its natural enemies. This could easily be true, particularly for those which become free of the host's cocoon and therefore lie exposed in the ground litter. An interesting question is, "How much jumping can be tolerated before there is insufficient energy remaining to allow complete development?" On the other hand, perhaps jumping increases the metabolic activity and aids in breaking diapause.

Habrocytus SP. AND *Dibrachoides druso.*—These pteromalids are rather large. Females are commonly 3 to 5 mm. long, and their bodies are a metallic bluish black. Males are somewhat smaller and of a different color in that iridescent green shows on the thorax and a broad transverse yellow band occurs on the abdomen. These two species can be distinguished by color differences in both sexes. Females of *Habrocytus* have black femora and nearly all white middle and posterior tibiae, and its males have a brownish antennal club. Females of *Dibrachoides* have dark amber or brown femora and tibiae, and its males have an orange-tan antennal club.

Although both species may develop as gregarious ectoparasites of mature larvae, prepupae, or young pupae of *Hypera brunneipennis*, laboratory indications are that *Habrocytus* prefers young pupae whereas *Dibrachoides* prefers mature larvae. Unmated females of both species produce only male progeny. Although *Dibrachoides druso* reproduces as a primary parasite on immature *Hypera*, the possibility that it might attack *Bathyplectes* (an endoparasite of *Hypera* larvae) was investigated with negative results. In repeated tests no secondary parasitic behavior was observed when placed with *Hypera* larvae known to contain larvae of *Bathyplectes*.

Further, because the *Bathyplectes* larva leaves the body of the *Hypera* larva soon after the latter forms its cocoon, there is small likelihood that *Bathyplectes* will suffer appreciably from the host-feeding and host-multilation behavior of *Dibrachoides* during this brief period. Because *Habrocytus* prefers later stages of *Hypera*, it is even less of a threat to *Bathyplectes*.

Both species paralyze the host with secretions issuing from the ovipositor. Attack by the females is deliberate, and violent movement of the host within its cocoon does not cause the parasite to lose interest. In fact, the gyrating host has been seen to impale itself on the parasite's ovipositor. Paralysis quickly occurs and either egg deposition or host feeding, or both, soon follows. *Dibrachoides* was observed feeding directly on exposed *Hypera* larvae. With her mandibles the female nipped a hole in the host and lapped up the exudate. Feeding from cocooned hosts requires construction of a feeding tube which takes several minutes to complete and is a very carefully performed ritual.

One biological difference between the two species is in the action of the paralyzing substance. Many hosts (*i.e.*, mature larvae) attacked by *Habrocytus* recover and complete their development, whereas host larvae paralyzed by *Dibrachoides* never recover. Paralyzed *Hypera* larvae exhibit a prominent sting mark where the ovipositor entered. Oviposition is somewhat slipshod in that the pteromalid female lays her ovipositor on the paralyzed host and deposits up to five eggs, some of which may fall off and lodge against the interior mesh of the host's cocoon. Development at 80° F. and 55% relative humidity is 12 days. Development at 70° F. and 60% relative humidity is 16 days. At pupation, several cupshaped meconia are voided. Their arrangement may be a characteristic of the genus.

The problem of diapause is also present with these species. Material reared from the Iranian shipments reproduced very well. The F_1 progeny, however, multiplied at a considerably lower rate and the F_2 generation increased at a still lower rate. As a final resort, the cultures were placed in a room maintained at 70° F. After 2 weeks of host feeding at this temperature, oviposition resumed. Explanations and refinements of technique are in progress, but in the meantime this empirical procedure saved the propagation program.

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Control of Millipedes in Greenhouse Soil[1]

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The millipede, *Oxidus gracilis* Koch, is well established in the southern and western United States and throughout the country in greenhouses. These arthropods have not been reported to be of much economic importance; however, their presence in large numbers often causes much apprehension to commercial growers as well as the home gardener. Little information is available concerning adequate control measures. Landis & Getzendaner (1959) described injury to potatoes by the millipede, *Blaniulus guttulatus* Bosc., and reported that DDT and BHC reduced populations and subsequent injury to potato tubers.

In 1959 and 1960 experiments were conducted to evaluate several insecticides for the control of heavy populations of *O. gracilis* Koch that had survived commercial applications of DDT, malathion, and BHC.[2]

METHODS.—The experiments were conducted in randomized blocks with four replications. Each plot consisted of a 25′ by 4′ section of a greenhouse bed planted to roses. A 3- to 4-inch corncob mulch had been maintained on all beds for several years, and more was added during these experiments.

All insecticides were applied at the rate of 5 pounds of active ingredient per acre. The treatments consisted of granulated Am. Cyanamid 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate) or heptachlor applied to the mulch, or ethion, dimethoate, or

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[2] The experiments were conducted with the cooperation of Akehurst Nurseries, Inc., Fullerton, Maryland.

Table 2.—Living millipedes[a] per sample from greenhouse rose plots treated with several insecticides at 5 pounds per acre, 1960.

Formulation	No. of Millipedes Alive After Weeks Shown					
	1	2	3	4	5	7
Ethion (emulsion)	14 a	3 a	4 a	3 a	4 a	1 a
Am. Cyanamid 18133 (granules)	36 bc	11 a	2 a	7 a	5 a	4 b
Dimethoate (emulsion)	24 ab	13 a	9 a	23 b	13 ab	14 bc
Parathion (wettable powder)	42 bc	29 b	27 ab	18 b	13 ab	8 b
Lindane (wettable powder)	52 c	31 b	22 ab	21 b	27 b	25 c
Untreated check	46 c	41 b	42 b	35 c	25 b	14 bc

[a] Means, within a column, not followed by the same letter are significantly different.

Table 1.—Living millipedes[a] per sample from greenhouse rose plots treated with several insecticides at 5 pounds per acre, 1959.

Formulation	Millipedes Alive After—	
	9 Days	18 Days
Ethion (emulsion)	4 a	3 a
Lindane (wettable powder)	32 ab	11 ab
Endrin (emulsion)	36 ab	14 ab
Heptachlor (granules)	63 bc	37 b
Untreated check	94 c	30 b

[a] Means, in a column, not followed by the same letter are significantly different.

endrin emulsions, or lindane or parathion suspensions applied at the above rate in 1,100 gallons of water per acre.

The millipede populations were estimated by counting all those in active living stages in samples of five trowel scoops of mulch or soil taken at random from each plot at different intervals after treatment. To facilitate examination of each sample the millipedes were separated from the soil and organic matter by successive screenings with ½- and ¼-inch hardware cloth and 14-mesh widow screen.

RESULTS—In 1959, ethion, lindane, and endrin reduced millipede populations (table 1). Ethion was highly effective for the duration of this experiment. Heptachlor was not effective.

In 1960, ethion gave effective control of millipedes for 7 weeks (table 2), dimethoate for 4 weeks. Am. Cyanamid 18133 was slower in its initial effect and at the first sampling results were poor. However, at subsequent samplings it gave effective control. Lindane and parathion were not effective.

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Metabolism of Injected Proline in DDT-Poisoned German Cockroaches[1]

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It has been briefly reported that DDT poisoning causes a decrease in the hemolymph proline concentration in *Periplaneta americana* (L.) (Corrigan & Kearns 1958). The other hemolymph amino acids are not significantly affected. The objective of our experiment was to confirm this remarkable biochemical effect of DDT and also to investigate the total free proline content and the rate of clearance of injected proline in both normal and DDT-poisoned *Blattella germanica* (L.).

Total body proline was determined by macerating single male cockroaches in 2 ml. of 20% methanol, heating nearly to the boiling point to denature proteins, and filtering the extract. The residue on the filter was washed with 2 ml. of 20% methanol and the combined filtrate adjusted to a volume of 5 ml. Aliquots of 0.5 ml. or 1 ml. were taken for analysis, using the relatively specific colorimetric method of Chinard (1952). This method uses ninhydrin in strong phosphoric-acetic acid and gives an intense red color (maximum at 515 mμ) with proline and ornithine, and possibly also labile peptides of these amino acids. The color was read quantitatively in a Bausch and Lomb Spectronic 20 colorimeter, by comparison with a standard curve for 40 and 80 micrograms of proline, in a final volume of 5 ml.

Two normal male cockroaches had total body proline content of 22 and 27 micrograms. Two cockroaches analyzed 12 hours after topical application of 20 micrograms of DDT had total body proline content of 6 and 7 micrograms. Only a fraction of the total free proline is in the hemolymph, most of it being in the body tissues. The data indicate that tissue proline, like hemolymph proline, is greatly decreased in DDT-poisoned cockroaches.

Table 1.—Total free proline content in male German cockroaches, at varying times after injection with 400 micrograms of L-proline.

Hours After Injection	Micrograms of Proline[a]	
	N	*DDT*
2	425, 428	264, 276
4	322, 304	
6	256, 260	176, 184
12		116, 152
24	88, 132	

[a] *N*, cockroaches not treated with DDT. *DDT*, cockroaches topically applied with 20 micrograms of DDT. The two numerical values given for each point represent results from two cockroaches, treated and analyzed separately.

Male cockroaches were injected with two microliters of a 20% solution of L-proline, using a Hamilton microliter syringe. This massive dose (400 micrograms of proline) caused no deaths, but the cockroaches were sluggish and weak for a few hours. At varying time intervals after injection, pairs of cockroaches were extracted for the proline analysis. A parallel experiment was carried out in which the cockroaches were topically applied with 20 micrograms of DDT immediately after the proline injection. The analytical results are shown in table 1. Clearance of proline

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is considerably faster in DDT-poisoned cockroaches, especially in the first few hours after injection. Whether the clearance is effected by excretion of proline or by its uptake and transformation by the tissues remains undecided, but it is hoped to solve this problem by studies using C^{14}-proline.

During the first few hours after injection the concentration of proline in the hemolymph is extraordinarily high. Paper chromatograms of droplets of hemolymph obtained by cutting off an antenna show enormous spots of proline. The rate of disappearance of proline from the hemolymph, indicated by the intensity of the proline spots on paper chromatograms of antenna hemolymph, seems to parallel the clearance of proline from the whole body. This suggests that there is rapid exchange of proline between hemolymph and tissues. The disappearance of proline is probably caused by metabolic transformation in the tissues, and the effect of DDT can be interpreted as a stimulation of this metabolic reaction by the muscular hyperactivity of poisoned cockroaches. The presence of abnormally high levels of proline in the body does not seem to cause a notable alleviation of the symptoms of DDT poisoning.

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Aerosol Dispensing System for Aircraft Disinsectization[1]

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For many years aircraft entering the United States have been treated with insecticide aerosols to prevent the introduction of undesirable insects. Treatments in flight have been made by the aircraft crew members, but to insure complete coverage government quarantine officials have applied recommended dosages of standardized aerosols (Fales *et al.* 1960) after the planes have landed and before passengers are allowed to leave.

The method commonly used to apply the aerosols in aircraft is by holding the valve of the aerosol container open and slowly walking through the fuselage. Passengers have been known to influence the crew members to discharge the unit in an isolated area such as a lavatory rather than throughout the fuselage. As a result very poor insect control has been obtained.

To overcome the disadvantages of the hand-discharged unit, an electrically activated system was designed by Yeomans *et al.* (1949) and was tested successfully by the Military Air Transport Service for more than 3000 flying hours. This system employed a timing device and a manifold made from aluminum tubing to supply the aerosol solution from a central container to small nozzles located throughout the aircraft. The method was not developed further because of the cost and weight of the installation.

To eliminate the need for the manifold piping system, a method has now been devised to discharge small one-shot aerosol containers simultaneously at strategic points throughout the aircraft. The containers usually are filled with sufficient insecticides and propellent to treat 1000 cubic feet of space. They are made from extruded aluminum and are 0.75 inch in outside diameter, 2.25 inches long, and have a capacity of 10 cc. A milled opening is provided at the end to clinch on a break-off tip valve after the unit has been filled with the aerosol solution. To eliminate the necessity for drilled discharge orifices with an expansion chamber, the siphon or eduction tube is made from small-bore polyethylene tubing. A tube 0.025 inch I.D. and 2 inches long is used for this size container (Fulton *et al.* 1950). A larger extruded aluminum container with a milled opening of the same size is available for use when higher dosages are required. This larger unit is $\frac{7}{8}$ inch I.D., $4\frac{3}{4}$ inches long, and has a capacity of 30 cc. An eduction tube 0.040 inch I.D. and 4 inches long is used for this container. These two containers, with additional eduction tubes and break-off tip valves, are shown in figure 1.

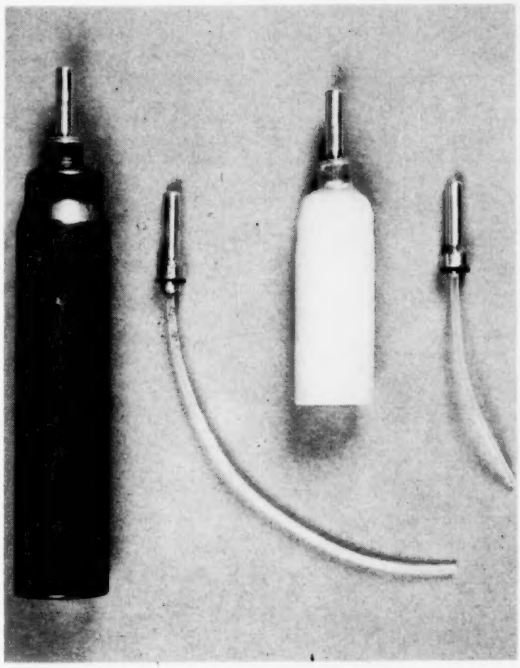

FIG. 1.—Two small aerosol containers (10-cc. and 30-cc.) with eduction tubes and break-off tip valves.

These dispensers, containing the desired dosage, are locked into position, and when empty are evidence to the quarantine officers that the dosage has been applied. The valves on the containers can be broken off by remote control by means of a spring mechanism at each dispenser, which can be released by an electric solenoid activated by a member of the aircraft crew using a central push-button switch or alternately by a lever-arm break-off system. These two types of break-off mechanisms are shown in figure 2.

Several insecticide formulations may be used in this type of unit. The limitation on concentration is about 5% of insecticide plus solvent in the propellent 12 (dichlorodifluoromethane). The number of units required to treat any space may be changed, depending on the concentration of insecticide used in the aerosol. Thus, if 100 milligrams of allethrin were required for 1000 cubic feet of space, and if a 5% allethrin formulation were used in 10-cc. units, each containing 10 grams of formulation, then one unit would be needed for each 5000 cubic feet. However, for uniform distribution of the insecticide in a long narrow space, such as an airplane fuselage, the aerosol should be released at several points. The total amount of insecticide for the given space would remain the same, but it should be divided into

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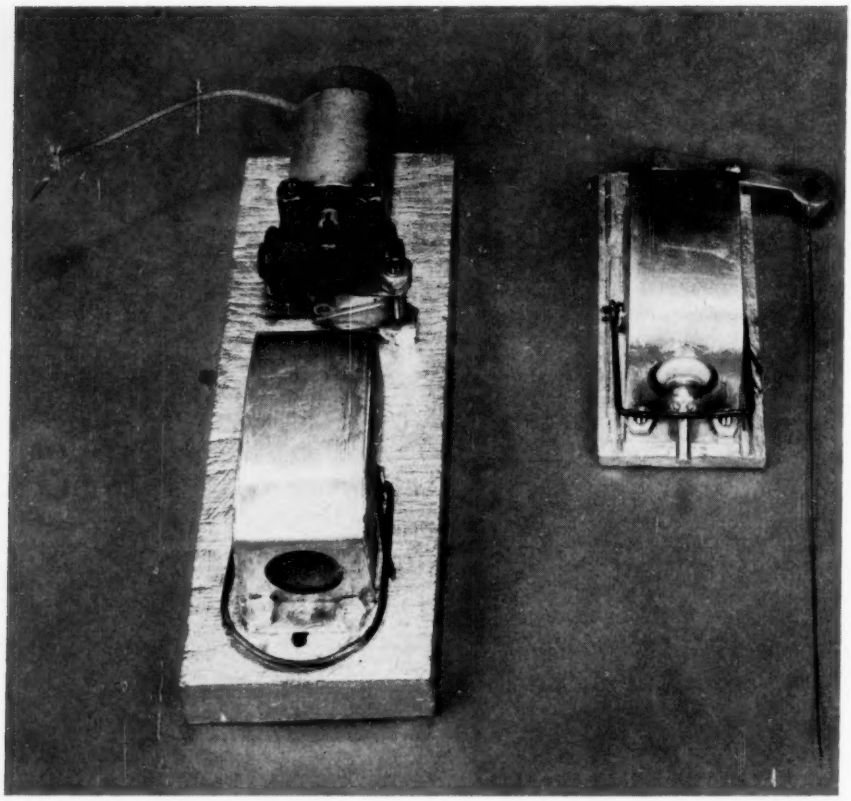

FIG. 2.—The solenoid, or electric, and the lever, or mechanical, mechanisms used to break off the tip of the 10-cc. aerosol container by remote control.

equal parts among the containers being used. The amount of propellent should be adjusted to make not more than 10 grams of total ingredients in each container, thus allowing for ample headspace.

One hundred per cent mortality of both caged and free-flying yellow-fever mosquitoes (*Aedes aegypti* (L.)) was obtained throughout a 2000-cu. ft. aircraft fuselage (on ground) at a rate of 100 mg. of allethrin per 1000 cu. ft. Two containers were used, each with 100 gm. of allethrin and sufficient propellent 12 to make 10 grams of total material.

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Evaluation of Insecticides for Control of the Tobacco Thrips on Peanuts[1]

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The tobacco thrips, *Frankliniella fusca* (Hinds) is the predominant species of thrips attacking Spanish peanuts in Frio County, Texas (Harding 1959). Feeding by these insects causes stunting of the peanut plants during May and early June. Control studies by Arant (1954), Arthur & Arant (1954), Arthur & Hyche (1959), Dogger (1956), Howe & Miller (1954), Poos (1945), Poos & Dobbins (1951), Poos *et al.* (1946), and Wilson & Arant (1949) for thrips on peanuts showed that BHC, DDT, toxaphene, and lindane were effective. The present study was conducted to compare the effectiveness of some of the more recently developed insecticides for thrips control on peanuts.

MATERIALS AND METHODS.—The tests were designed as randomized blocks in which the treatments were replicated three times. The plots were three rows wide and 35 feet long. Two rows were treated on May 9, 1960 when the plants averaged 5 inches in height in each plot leaving one untreated guard row between plots. All treatments were applied as sprays using one

[1] Technical Contribution No. 3625 Texas Agricultural Experiment Station Accepted for publication September 26, 1960.

Table 1.—Chemicals, dosages, and per cent control of thrips on peanuts, 1960.

TREATMENT	POUNDS ACTIVE INGREDIENT PER ACRE	PER CENT CONTROL[a] (DAYS AFTER APPLICATION) 3	8
Experiment 1			
Trithion	1	97.6 a[c]	84.9 a
Methyl Trithion	1	95.6 ab	78.8 a
Diazinon	0.5	89.3 b	52.2 c
Dylox	1	73.2 c	68.4 b
Perthane	1	64.2 d	68.3 b
Check[b]		8.4	23.2
Experiment 2			
Sevin	1	93.0 a	59.5 b
Ronnel	0.75	89.1 a	48.6 bc
Ethion	0.5	84.7 a	82.4 a
Dibrom	0.75	85.5 a	38.5 c
Thiodan	0.75	68.3 b	60.2 b
Check[b]		8.5	23.7
Experiment 3			
Malathion (SF-60)	1.25	99.2 a	66.1 c
Dimethoate	0.5	98.8 ab	79.3 b
Malathion (Standard)	1.25	97.5 ab	67.3 c
Phosphamidon	0.5	94.5 b	16.7 d
SD 4402	0.25	87.7 c	91.3 a
Check[b]		7.8	11.4
Experiment 4			
Phosdrin	0.5	98.2 a	24.9 c
Parathion	0.5	98.9 a	84.1 a
Guthion	0.5	97.8 a	54.6 b
DDT	1.5	97.1 a	93.7 a
Toxaphene	1.5	93.8 a	91.4 a
Check[b]		9.1	14.6

[a] Based on untreated check plots.
[b] Mean number of thrips per terminal leaf cluster.
[c] Treatments having a common letter for a given column are not significantly different at the 5% level of probability. Superiority follows the alphabet, in that *a* is superior to *b* and *d* is the most inferior.

nozzle per row at the rates indicated in the tables. Five gallons of spray mixture were applied per acre at a pressure of 30 p.s.i.

The treatments were evaluated by counting adult and larval thrips in each plot on 10 randomly selected terminal leaf clusters which were made up of four folded leaflets. Records were obtained 3 and 8 days after application. The average number of thrips per terminal leaf cluster in the untreated plots is shown in the table. The data were analyzed statistically and Duncan's New Multiple Range Test (1955) was used to evaluate differences between treatment means.

The insecticides included in these tests were DDT, toxaphene, parathion, dimethoate, ronnel, ethion, malathion, phosphamidon, Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3,-benzodioxathiepin-3-oxide), Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3,-benzotriazine-(3-4*H*)-ylmethyl phosphorodithioate), Perthane® (1,1-dichloro-2,2-bis (*p*-ethylphenyl)ethane), Diazinon® (*O,O*-diethyl*O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), Dibrom® (1,2-dibromo-2,2-dichloroethyl) dimethyl phosphate), Trithion® (*S*-(p-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), Methyl Trithion® (*O,O*-dimethyl *S*-(*p*-chlorophenylthio) methyl phosphorodithioate), Dylox® (dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate), Phosdrin®, (1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate), Sevin® (1-napthyl methyl-carbamate), and Shell Development Compound 4402 (1,3,4,5,6,7,8,8-Octachloro 3*a*, 4,7,7*a*-tetrahydro-4,7-menthanophthalan).

RESULTS AND DISCUSSION.—Table 1 presents the results of four experiments. In the first test, Trithion and Methyl Trithion were the most effective chemicals 3 days after application. Methyl Trithion, however, did not differ significantly in control from Diazinon. Dylox was superior to Perthane but both materials were less effective than Trithion or Methyl Trithion. Data obtained 8 days after treatment showed that Trithion and Methyl Trithion were most effective. Dylox and Perthane were superior to Diazinon.

The data from experiment 2 indicated that Thiodan was less effective than the other chemicals 3 days after treatment. Records 8 days after treatment showed that ethion was still effective and Thiodan, Sevin, and ronnel were next in order of effectiveness.

Data from experiment 3 showed that control obtained with malathion and an additive termed SF-60 3 days after application did not differ significantly from that obtained with malathion (agricultural grade) or dimethoate which, in turn, did not differ from phosphamidon. Shell Development Compound 4402 was inferior to the other chemicals. Eight days after treatment, SD 4402 provided the most satisfactory control and dimethoate was next in effectiveness. Residual control by phosphamidon was decidedly inferior.

Records obtained 3 days after application in experiment 4 indicated that all insecticides were equally effective. Data taken 8 days from treatment showed that toxaphene, DDT, and parathion were superior in effectiveness to Guthion and that Phosdrin was relatively ineffective.

In conclusion, Trithion, Methyl Trithion, ethion, SD 4402, toxaphene, DDT, and parathion were the most satisfactory chemicals for thrips control on peanuts at the rates tested. Residual control with Dibrom, phosphamidon, and Phosdrin was relatively poor.

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Effect of Gamma Radiation on the Reproductive Potential of the Mexican Fruit Fly[1]

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The eradication of the screw-worm (*Callitroga hominivorax* (Cqrl.)) on the island of Curacao and in the Southeastern States by overflooding natural populations with flies made sterile by exposure to gamma radiation demonstrated the feasibility of the sterilization method of insect control (Baumhover *et al.* 1955, Knipling 1955, 1959, Lindquist 1955). Studies in Hawaii revealed promise for application of the method to polygamous tropical fruit flies (Steiner & Christenson 1956, Christenson 1956). The possible usefulness of the method to prevent or curtail the incipient infestions responsible for control and regulatory problems in citrus areas in Texas and along the California-Mexico border, or those that may develop elsewhere within our country, suggested the need for similar research on the Mexican fruit fly (*Anastrepha ludens* (Loew)).

Two cobalt-60 sources rated at 800 and 1300 curies each were used to irradiate puparia of the Mexican fruit fly. These were made available through the cooperation of the Instituto Mexicano del Seguro Social[2] and the Sociedad de Beneficencia Española.

In preliminary experiments initiated in October 1958, there was no further development when 4-day-old puparia were exposed to dosages above 2,000 r. Within the 7,000 to 12,000 r exposure range, emergence from puparia treated when they were 8 days old was approximately 20% to 60% lower than from untreated pupae. Emergence from puparia treated when 12 days old, or about 4 days before they would emerge at 77° F., appeared to be normal at all radiation levels from 1,000 to 12,000 r. The rate of exposure was less than 100 r per minute in these tests.

Adults of both sexes were rendered sterile when 12-day-old pupae were exposed to 5,000 r (table 1). The irradiated females did not develop eggs. Both sexes remained sterile until death occurred, sometimes as long as 107 days after emergence, and longevity appeared to be normal. Neither mating behavior nor aggressiveness of young treated males were adversely affected by this dosage. A latent effect on mating behavior was apparent, however, 30- to 39-day-old males being able to mate first only 59 times compared with 141 times for normal flies in competition trials.

The rate sensitivity of the Mexican fruit fly was also investigated within limits possible with the available equipment. In 1,000 r increments, dosages of 1,000 r to 5,000 r were applied to 12-day-old puparia at rates of 90 r, 70 r, 50 r, 30 r, and 10 r per minute at each level.

Table 1.—Effect of gamma radiation on reproduction of the Mexican fruit fly.[a]

Dosage (r)	Normal Males Mated with Irradiated Females		Irradiated Males Mated with Normal Females	
	Number of Eggs	Per Cent Hatch	Number of Eggs	Per Cent Hatch
1,000	3,270	73.1	3,793	66.7
2,000	17	23.5	3,229	2.4
3,000	0	0	2,673	.1
4,000	184	0	4,151	0
5,000	0	0	4,824	0
Untreated=3,631 eggs, 73.6% hatch				

[a] 50 pairs for each treatment.

Table 2.—Effect of variation in rate of gamma radiation on reproduction of the Mexican fruit fly.[a]

Dosage and Rate per Minute (r)	Normal Males Mated with Irradiated Females		Irradiated Males Mated with Normal Females	
	Number of Eggs	Per Cent Hatch	Number of Eggs	Per Cent Hatch
2,000 r				
90	68	0	5,202	3.8
70	0	0	8,697	1.8
50	57	28.1	7,516	5.3
30	180	44.4	8,936	9.2
10	1,768	20.3	4,325	12.0
Untreated=7,003 eggs, 48.6% hatch				

[a] Four replicates of 25 pairs for each exposure and five replicates for controls.

Table 3.—Influence of sterile flies (5,000 r) on the fertility of eggs produced by normal laboratory populations of the Mexican fruit fly.

Fly Population[a]		Number of Eggs	Per Cent Hatch
Sterile	Normal		
		Series 1	
50	50	1,829	55.5
250	50	1,898	15.8
500	50	1,511	17.3
Untreated	150	1,772	61.4
		Series 2	
60	60	1,402	33.9
300	60	834	47.2
600	60	1,880	43.1
3,000	60	1,259	0.0
Untreated	300	1,132	74.1
		Series 3	
60	60	2,026	50.6
600	60	2,380	24.9
1,500	60	2,969	6.8
3,000	60	2,250	0.7
Untreated	180	2,926	85.8

[a] Equal numbers of both sexes.

At the critical 2,000 r dosage, as the dose rate increased irradiated females deposited fewer eggs and irradiated males became less fertile. These trends are illustrated in table 2. Females receiving more than 2,000 r developed no eggs and the number of viable eggs deposited by untreated females mated with irradiated males gradually diminished until complete sterility was obtained at 5,000 r.

In overflooding tests with caged fly populations there were marked reductions in the fertility of eggs, despite the frequent mating habits of the females when sterile flies dominated normal individuals at ratios of 5, 10, 25, and 50 to 1 (table 3). The almost total effect at the 50 to 1 overflooding ratio was of unusual interest.

Field releases in semi-isolated Mexican fruit fly areas in Morelos, Mexico, have been initiated. As yet these have not progressed to the level where population suppression may be expected.

[1] Accepted for publication October 3, 1960.
[2] Irradiation of puparia at the Instituto Mexicano del Seguro Social was done under the supervision of Ing. Jorge Halvas.

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Field Tests with New Cattle Grub Systemics[1]

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Several papers have been published on the effectiveness of Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) against migrating cattle grub larvae (*Hypoderma lineatum* (DeVill.) and *bovis* (L.)) since the work by Brundrett *et al.* (1957). Although Co-Ral is presently the only recommended systemic spray treatment for grubs, a new material Ruelene® (4-*tert*-butyl-2-chlorophenyl methyl methylphosphoramidate) was reported by the Dow Chemical Company (1959) to be effective when applied either externally or administered internally. Further tests with Ruelene were reported by Swanson & Collier (1960) and Bracewall & Schurr (1960). The promising systemic properties of Ruelene have been shown in a variety of tests made during the last 2 years by workers of the Corvallis, Ore. and Kerrville, Tex. laboratories of the Entomology Research Division (unpublished data).

During the fall of 1959 preliminary field trials were run with Ruelene, Bayer 29493 (*O,O*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate), and Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate). Co-Ral was included for comparison in one of the main experiments. This paper presents the results of those tests.

MATERIALS AND METHODS.—The materials were tested as commercial formulations and prepared from 25% Co-Ral and Ruelene wettable powders and 50% Bayer 22408 and Bayer 29493 wettable powder and emulsion concentrate, respectively. They were applied at an average rate of 1 gallon per animal with a Bean power sprayer operating at 250–300 p.s.i., and equipped with a No. 785 adjustable spray gun with a No. 6 disk.

The cattle were all short yearlings to 2-year-old Herefords, located in the Willamette Valley of the state. They were treated in October and November, several weeks before grub encystment. The control animals were not separated from the treated ones. Examinations were made in January, February, and/or April. The grubs present in January and most of those found in February were *lineatum*. A small number of those checked in February and all those removed in April were *bovis*.

RESULTS AND DISCUSSION.—The data obtained with the various spray treatments are presented in table 1. In Herd 1 Bayer 29493 was tested at 0.25%, Ruelene and Bayer 22408 at 0.75%, and Co-Ral at 0.5%. Twenty head, selected at random, were treated with each compound and 12 were used as controls. Bayer 29493 gave perfect control; not a single grub was found in any of the animals. Excellent control (98% to 100%) was also obtained with Ruelene. Co-Ral caused only a 75% to 86% kill of grubs and Bayer 22408 was relatively ineffective (0% to 40%). The poor results obtained with Bayer 22408 were

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Table 1.—Systemic control of cattle grubs with insecticide sprays applied in the fall of 1959 at the rate of 1 gallon per animal.

Insecticide and Per Cent of Active Ingredient	Number of Animals in Test	Effectiveness of Treatment					
		January		February		April	
		No. of Grubs	Control (%)	No. of Grubs	Control (%)	No. of Grubs	Control (%)
			Herd 1				
Bayer 29493, 0.25	20	0	100	0	100	0	100
Ruelene, .75	20	0	100	0.2	98	0.15	98
Co-Ral, .50	20	3.7	75	2.8	76	.9	86
Bayer 22408, .75	20	8.7	40	8.2	29	6.7	0
Controls, —	12	14.6	—	11.6	—	6.6	—
			Herd 2				
Ruelene, .25	12	2.9	79	3.6	74	1.7	73
.5	12	.16	99	1.0	93	.66	90
.75	12	1.2	91	2.3	83	.58	91
1.0	12	0	100	.16	99	.08	99
Controls, —	11	13.5	—	13.9	—	6.3	—
			Herd 3				
Ruelene, .25	30	0	100	.5	92		
.75	16	0	100	0	100		
Controls, —	14	.5	—	6.2	—		
			Herd 4				
Ruelene, .5	29	0	100				
Controls, —	16	5.2	—				

not unexpected since it had shown little promise in screening tests.

The remaining three herds were sprayed with various concentrations of Ruelene. In Herd 2 the material caused only fair kill (73% to 79%) of grubs at 0.25%. Much better control (83% to 99%) was obtained with the 0.5% to 0.75% sprays. Although the 1% spray gave 99% to 100% control, it is of interest that a few grubs escaped this high concentration. Excellent control with Ruelene was indicated in Herds 3 and 4, although the animals were not as heavily infested and fewer examinations were made.

Based on the results of the above tests and past experience with systemic insecticides, the conclusion that at least a 0.5% concentration of Ruelene would be needed to consistently produce the type of control needed seems justified. Further research to determine the effectiveness and limitations of Bayer 29493 is obviously warranted.

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Effectiveness of Insecticides against the Rusty Plum Aphid and Ants[1]

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Sugarcane mosaic was considered one of the major factors responsible for sugarcane failures in Louisiana in the mid twenties. Until recently the disease was kept under control by the use of resistant varieties. However, with the release of two high-yielding susceptible varieties for commercial culture and the development of a new strain of the virus, this disease has again become of economic importance (Abbot 1958). It is now considered the most serious disease confronting the Louisiana sugar industry and has renewed interest in the problem (Forbes & Steib 1960).

Although the rusty plum aphid (*Hysteroneura setariae* (Thos.)) is the least efficient of the four known vectors, it is probably the most important in Louisiana because of its greater abundance on sugarcane. Heavy local infestations of this aphid developed in the southeastern part of the cane belt during 1959.

Previous investigations (Ingram *et al.* 1939) have shown that a close association exists between the rusty plum aphid and ants, especially the Argentine ant (*Iridomyrmex humilis* Mayr). The ants move the aphid from one sugarcane plant to another, from sugarcane to alternate hosts in and around cane fields, and vice versa. They often build protective dirt and borer frass coverings over the aphid colonies to protect them against natural enemies and rains.

As a possible means of developing a program of decreasing mosaic spread by vector control with chemicals, two experiments were conducted at Raceland and Mathews, La., to evaluate the effectiveness of several insecticides for the control of the rusty plum aphid and ants associated with them. Ratoon fields of the mosaic-susceptible variety N. Co. 310 sugarcane growing on well-drained sandy soil and with heavy and uniform populations of the insects were selected for the tests. The experiments were of a latin-square design with six replications of each of five insecticides and of the control. Each plot consisted of a single row of cane, 25 feet long, separated by 12-foot buffers. Heptachlor, demeton, Trithion® (*S*-(p-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), and malathion were applied at 1 pound of active ingredient per acre in emulsions containing 30 gallons of water with a hand air-compressed sprayer. Dimethoate was applied at 1-pound and ½-pound rates. Applications were made on May 13 and June 3.

Table 1.—Control of aphids and ants with various insecticidal sprays.

Insecticide and Rate of Application (pounds per Acre)	First Experiment		Second Experiment	
	Aphid-Free Plants (%)	Reduction of Ant Population (%)	Aphid-Free Plants (%)	Reduction of Ant Population (%)
Heptachlor 1	96	96	—	—
Demeton 1	89	89	88	84
Trithion 1	87	84	86	77
Dimethoate 1	83	84	84	80
0.5	—	—	84	78
Malathion 1	79	74	76	65

The number of aphid-infested plants and actual number of ants were determined in a 10-foot sample in each plot at weekly intervals between May 15 and June 26. The data are summarized in table 1.

An indication of the toxicity of the different insecticides to the aphid was obtained 48 hours after treatments. The number of plants with colonies of dead aphids still clinging to the collar lobes were as follows: for demeton, 15; dimethoate at the 1-pound dosage, 11; Trithion, 9; dimethoate at ½ pound, 6; malathion, 5; and heptachlor, 3.

These experiments show that all the insecticides tested were highly effective against the aphid and ants, as indicated in table 1.

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Effect of Captan upon Reproduction in the Two-Spotted Spider Mite, *Tetranychus telarius*[1]

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This paper presents the results of preliminary investigation on the effect of the fungicide captan upon reproduction in the two-spotted spider mite, *Tetranychus telarius* (L.) (Acarina: Tetranychidae). The work was undertaken to determine if this

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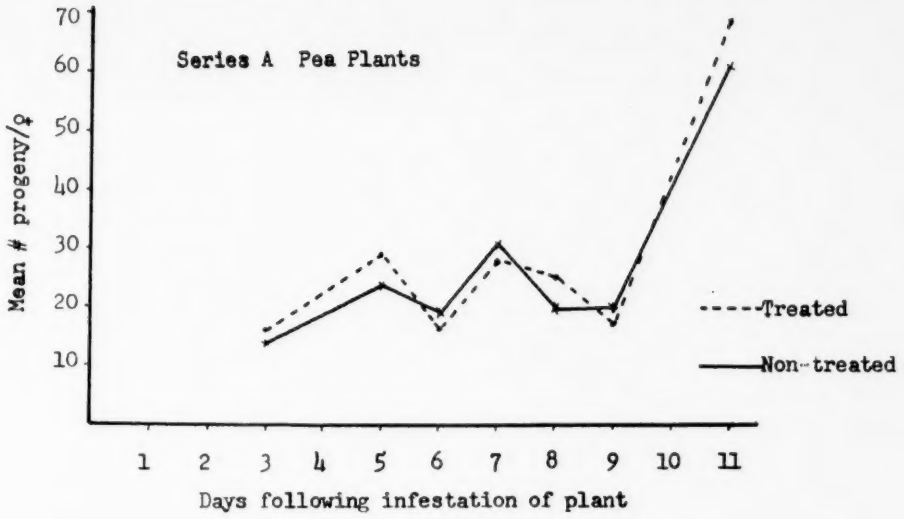

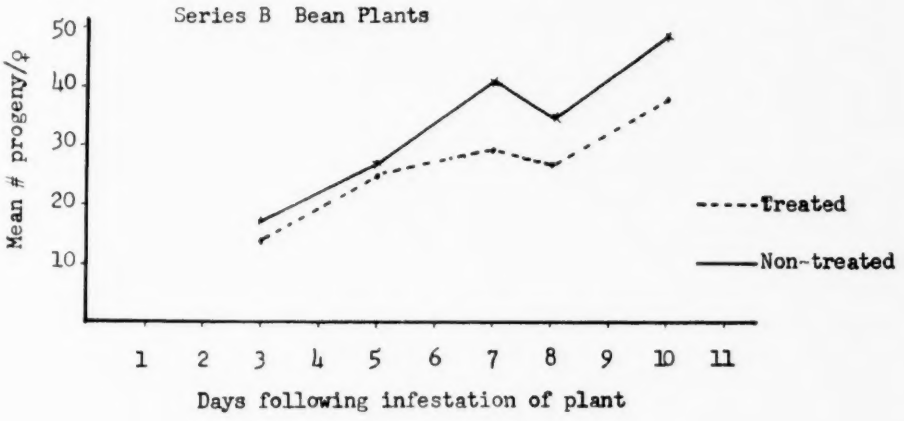

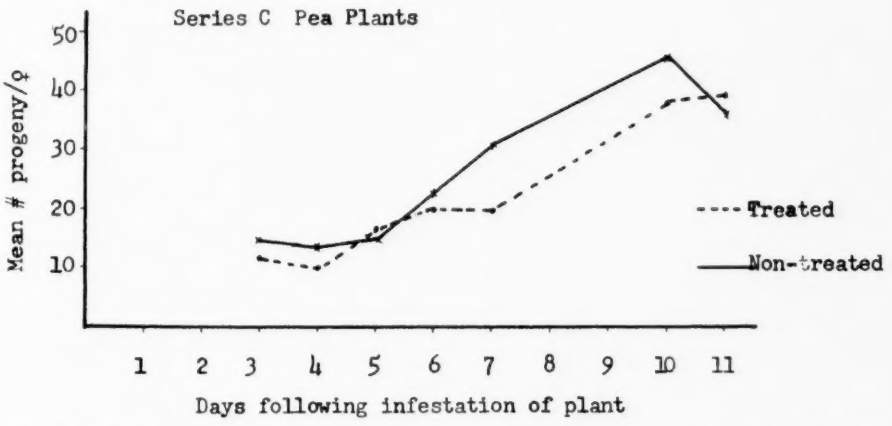

FIG. 1.—Results for the three series of experiments showing the mean number of progeny (eggs and mites) produced per parent female for particular days following plant infestation for captan-treated and nontreated plants.

chemical could be associated with population build-ups of spider mites occurring in some orchards in Georgia.

It is well substantiated in the literature that the use of certain insecticides results in population increases in some of the Tetranychid mites (Locher 1958, Oatman 1959, Klostermeyer 1959, Michelbacher 1959). The chemicals reported in the literature as associated with increases in mite populations include, among others, DDT, Sevin® (1-naphthyl methylcarbamate), dieldrin, endrin, and Tedion® (2,4,4',5-tetrachlorodiphenyl sulfone). To date, very little information is available as to the effect of fungicides or many other commonly used chemicals upon mite reproduction. Information of this nature should be of value in considering recommendations for control.

In the work reported here, three series of laboratory tests were set up to note the effect of captan upon reproduction in the two-spotted spider mite. In series A and B, which were run simultaneously, captan was used at the rate of 2 pounds of 50% captan per 100 gallons of water. In series C, captan was used at the rate of 4 pounds per 100 gallons of water. In all tests the following procedure was used: leaves of the host plant (lima beans or peas) were dipped in the captan solution and allowed to dry; then a known number of adult female mites, usually five, was transferred to a treated leaf by use of a small brush. Mites were also reared simultaneously on nontreated plants. At various intervals, following leaf treatment, all leaves from a single plant were removed, and the offspring (eggs and mites) on each leaf were counted. The leaves were held in a refrigerator before counting to reduce the activity of the mites. A total of from 300 to 400 parent female mites were used in each series of tests, and the smallest number of offspring for any one series was more than 7,000. The age of the parent female mite was not constant in these tests. Mites reared on pea plants were transferred to treated pea plants; mites from bean plants were transferred to treated bean plants. Plants for any single test were of the same age. Only pea plants were used in series C. These tests were carried out at a temperature of 85° F. in an insectory under artificial lighting.

The results of these tests, presented in figure 1, show no striking effect of captan upon the population structure of the mites. In series B and C, there is an indication of a reduction in the average number of offspring from females kept on treated as compared with nontreated leaves; however, this relationship is not constant for series A. The data indicate that captan has a slight inhibiting effect, if any, upon the mite population under the conditions of this study. The general reduction in offspring noted on the treated leaves in series B and C was caused, in each case, by a lower egg production and not by embryonic or preadult mortality as the per cent of eggs was about the same in all cases. No delay in egg hatching or egg mortality was noted on treated as compared with nontreated leaves. In a subsequent test in which mites were reared from egg to adult on captan-treated leaves, no effect on the population, when compared with the controls, was noted in the F_1 generation.

The graphs of figure 1 give some information on the accumulative population growth of this mite. In all cases, food was still plentiful at the time the offspring were counted. Based on the total population number, *i.e.*, eggs and mites, in the different series, 58% to 66% of the population consisted of eggs. Almost no immature mites were found in the counts made 3 days after the parent females were put on the leaves; hence, it would seem unlikely that second generation females contributed to the egg production. (Some second-generation females may have been laying in series A by the 11th day.) Analysis of the egg production data showed that in most cases there was almost no decrease in fecundity from the 3rd to the 11th day. An exception is series A where there was some decline in fecundity which is reflected in the graph of the total offspring for that series. Since a few of the parent females may have been up to 7 days old at the start of the test, this would mean that some of the females had been reproducing for about 15 days by the termination of the study without showing a noticeable slackening in fecundity.

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Occurrence of *Hippelates* Eye Gnats in a Coastal Habitat in California[1]

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Hippelates gnats, which are believed to be the vector of acute conjunctivitis in the Coachella Valley, are significant pests in many of the cultivated desert areas of Southern California. They are also becoming more of a nuisance in certain agricultural areas of San Joaquin Valley. The need for gnat control resulted in the formation of the Coachella Valley Mosquito Abatement District in 1928. More recently, with the development of agriculture in Borrego Valley, a former desert waste area in eastern San Diego County, this problem assumed such proportions that an abatement district was also organized there in 1955.

In these desert areas adult gnats emerge in great numbers from the irrigated and cultivated soils during the hot summer and fall months and decline in numbers during the colder winter months. The observations reported here are concerned with the occurrence of *Hippelates* gnats in large numbers in a markedly contrasting habitat, that of the mild coastal area of San Diego County known as Rancho Santa Fe.

Rancho Santa Fe is located 4 miles from the Pacific Coast. It is hilly, consisting of approximately 600 residential-agricultural estates, with a total area of about 9 square miles. There are an estimated 1,500 inhabitants. Agriculture on the estates is devoted to citrus and some truck crops. Large acreages of truck crops are grown in the San Dieguito Flood Plain which borders this area to the south. Many estates have one or more acres of citrus. *Eucalyptus* and chapparal constitute the primary cover in the uncultivated areas. A golf club course, 1.2 miles long, lies in the center of the Rancho.

Adult *Hippelates* traps, designed by Dr. Ernest R. Tinkham, former manager of the Coachella Valley Mosquito Abatement District, were located at nine different places in or adjacent to Rancho Santa Fe.[4] The traps were baited with rotten eggs, using the procedure described by Mulla (1958), and the gnats were collected, sorted, and counted weekly. Three traps were placed on the golf course where many complaints originated. One was placed near a dairy located on the San Dieguito River bottom, and the remaining five traps were distributed throughout the estates.

[1] Presented at Pacific Branch Meeting of the Entomological Society of America, June 25, 1958, San Diego, California. Accepted for publication October 10, 1960.

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[4] We gratefully acknowledge the cooperation of Rancho Santa Fe Association for constructing and maintaining the traps, and of Dr. Mir S. Mulla, University of California, Citrus Experiment Station, Riverside, who assisted in certain technical aspects of this study.

Table 1.—*Hippelates* spp. taken in weekly collections from nine traps at Rancho Santa Fe, San Diego County, California

1957–1958

MONTH AND YEAR	HIGHEST VALUE	LOWEST VALUE	MEDIAN	NO. OF COLLECTIONS
1957				
February	76	2	24	27
March	1,813	1	46	35
April	7,410	1	234	35
May	5,029	39	480	34
June	6,048	2	428	34
July	6,000	10	334	42
August	4,750	0	277	36
September	2,680	0	97	45
October	1,278	0	16	34
November	234	0	7	35
December	260	0	18	36
1958				
January	1,140	0	122	36
February	379	3	34	17

RESULTS AND DISCUSSION.—Traps collected three *Hippelates* species: *H. collusor* Townsend, *H. robertsoni* Sabroski and *H. pusio* Loew. *H. robertsoni* predominated during the winter months, and *H. collusor* was more common in the summer months. *H. pusio* occurred only in small numbers in a few traps in January and February.

Results of weekly gnat counts for a full year are presented in table 1. The data indicate that *Hippelates* gnats were present all year in Rancho Santa Fe, with largest numbers occurring during the warmer months. During the winter there was enough gnat activity to bother golfers occasionally. The high late spring and early summer counts appear to be associated with the period of extensive soil cultivation, large scale emergence occurring just a few weeks after cultivation. This is consistent with laboratory data obtained by Burgess (1951) who obtained emerging adults within 21 days following egg deposition. The high and low gnat counts for each month demonstrated the extreme variability between collections. Even during the months of relatively high gnat counts, such as July, there were some extremely low counts. Such diverse data probably reflected local emergence of adult gnats from sources close to individual traps.

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Parasitization of *Cactoblastis cactorum* by *Trichogramma semifumatum* in Hawaii[1]

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In April 1950, *Cactoblastis cactorum* (Berg), a phycitid moth endemic to South America, was released on the island of Hawaii in an attempt to control the prickly pear cactus, *Opuntia megacantha* Salm-Dyck (Fullaway 1954). Within 5 years, the moth became established on all the major islands. It has killed many cactus plants in zones with less than 20 inches of rain per year, but has not been as effective in regions with higher rainfall.

Van Zwaluwenburg (1954) reared wasps of the genus *Trichogramma* from eggs of *C. cactorum*. Although other species of *Trichogramma* had been regarded as no more than incidental enemies of *C. cactorum* in Australia (Dodd 1940, p. 152) and South Africa (Pettey 1948, p. 86), it was considered desirable to find out if the lower effectiveness of *C. cactorum* in wetter cactus areas was consistently associated with high levels of parasitization by *Trichogramma semifumatum* (Perkins).[3]

During the periods from October 1955 through June 1956 and from May through July 1960, the bionomics of *C. cactorum* and of *T. semifumatum* were studied in the laboratory. There were usually from 30 to 100 eggs in each of the three or four stick-like clusters laid by a female of *C. cactorum*. Eggs of *C. cactorum* hatched about 4 weeks after oviposition. When the incubation period was 28 days, the eggs were most susceptible to attack by *T. semifumatum* during the first 7 days and could not be parasitized during the last 4. Three to five days after attack, parasitized eggs turned black. They remained black when, 9 to 14 days after attack, two or three adult parasites emerged from each egg. The maximum number of eggs parasitized by one *T. semifumatum* female was 20.

Parasitization of *C. cactorum* by *T. semifumatum* was measured in three areas on Oahu: Waahila Hill, Saint Louis Heights, and Diamond Head. The annual rainfall in the first two areas was usually about 30 inches but Diamond Head generally received less than 20 inches. Destruction of prickly pear cactus by *C. cactorum* has been most conspicuous in the Diamond Head area.

Two techniques, exposure and collection, were used in measuring parasitization. The exposures were made in two series: from December 23, 1955 to January 15, 1956 and from February 3 to February 26, 1956. In both series, freshly laid egg sticks from laboratory colonies were placed on selected cactus cladodes each week for 3 subsequent weeks. By exposing egg sticks only when they were most susceptible to attack, it was possible to detect the presence of *T. semifumatum* at any time during the 3-week series. During the first series, 3,809 eggs were recovered and 1.6% were found to be parasitized by *T. semifumatum*. In the second, 19.9% of the 3,114 eggs recovered had been attacked. The greatest increase, from 0% to 37.8%, occurred at Diamond Head. This rise in parasitization, however, had little effect on the survival of *C. cactorum*, since the moth laid few eggs in the cactus areas during February. Apparently the populations o *T. semifumatum* increased on the eggs of other lepidopterans in the areas.

Data from collections also indicated that parasitization by *T. semifumatum* was a minor source of mortality in populations of *C. cactorum*. Samples were taken intermittently during the period from December 1955 through July 1960. The wasp had attacked 2.0% of the 10,611 eggs collected at Diamond Head and 7.5% of the 9,073 eggs gathered at Saint Louis Heights and Waahila Hill. It may be concluded that parasitization by *T. semifumatum* was higher in the wetter areas but not high enough to greatly reduce the effectiveness of *C. cactorum*.

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[1] Accepted for publication October 13, 1960.

[2] Portions of this note are based on the author's thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at the University of Hawaii.

[3] Identified from living material sent to Dr. W. Quednau at the Biologische Bundesanstalt für Land- und Fortswirtschaft, Berlin-Dahlem.

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Control of Cattle Grubs and Horn Flies by Summer Dipping with Co-Ral[1]

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Effectiveness of Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) as a systemic cattle grub control is well recognized. Dermal applications of Co-Ral in controlling larvae of the common cattle grub, *Hypoderma lineatum* (De Vill.), were first reported by Brundrett *et al.* (1957). Drummond (1959) found that a single spraying of 0.5% and the repeated sprayings of 0.75% were both almost 100% effective in preventing grub encystment, but the single application of 0.25% spray failed to give complete control. The method of application in these tests has been by spraying. The concentration shown to be nearly completely effective is 0.5% and this is the currently recommended method and rate of application. At 0.25% as a single application, it has not been satisfactory. Brundrett *et al.* (1958) showed that Co-Ral is also toxic to horn flies, *Siphona irritans* (L.). Alford & Jones (1959) showed that a spray of Co-Ral at 0.25% was effective 2 to 4 weeks against horn flies.

This field study was designed to determine the following: (1) the effectiveness of 0.25% Co-Ral wettable powder against horn flies and cattle grubs when used as a dip, (2) the rate of removal of insecticide from the vat, and (3) whether the concentration left would tend to fluctuate upward or downward.

METHODS AND MATERIALS.—A 2,000-gallon capacity vat with no cover was used. Fifteen hundred gallons of water were pumped into the vat and then the insecticide, 30 pounds of actual Co-Ral from wettable powder 25% active material, was added. A concentration of 0.25% was desired, but because of the lack of available Co-Ral at time of charging only 0.24% concentration was obtained.

The material in the vat was agitated before each dipping by sinking a 5-gallon pail with rope attached and rapidly lifting and releasing it, creating a churning action.

Immediately following mixing and prior to any dipping, a 1-quart sample was taken. In each instance after 25 animals were dipped, another quart was taken. These samples were analyzed by the Chemagro Corporation. Between the second and third dippings, heavy rainfall added 300 gallons of water to the vat. To bring the concentration back to 0.24%, 8 pounds of Co-Ral were added.

Five herds were used in this study with the dipping vat centrally located in relation to them. One of these herds, used as a check for horn fly counts, received no insecticide during the duration of the study. This herd was located near the center of the area in which the four treated herds were located. The animals used for checks in grub control studies were untreated animals scattered throughout the four treated herds. Of the four herds dipped, three were dipped three times and the other was dipped twice.

The initial fly counts were made 24 hours following each dipping; thereafter the counts were made at weekly intervals.

Table 1.—Effect of dipping cattle with Co-Ral for control of horn flies, 1959.

No. of Head		Date of Count	Average Number of Flies per Head	
Treated	Untreated		Treated	Untreated
		First dipping (June 18)		
123	30	June 19	0.8	186.6
		June 26	8.09	161.6
		July 3	12.3	125.7
		July 10	34.3	191.0
		Second dipping (July 17)		
97	28	July 18	0.55	161.6
		July 24	18.3	274.3
		July 30	No count	No count
		Aug. 6	167.7	262.5
		Third dipping (August 20)		
101	22	Aug. 21	0.5	188.0
		Aug. 28	22.7	190.0
		Sept. 4	85.4	246.5
		Sept. 11	37.2	220.5
		Sept. 18	97.4	242.3
		Sept. 26	170.7	240.5

Table 2.—Effect of dipping cattle with Co-Ral for control of *Hypoderma lineatum*.

No. Animals Examined	No. Times Dipped	No. Grubs per Head		Total Grubs
		Treated	Untreated	
69	3	0.015		1
17	2	0.29		5
12	0		5.3	64

RESULTS.—In table 1 the results obtained against horn flies are shown.

Total rainfall during the period following the first dipping was 0.64 inches and the treatment was still fairly effective, as compared with the untreated check, after 3 weeks. Beginning on July 17, the day of the second dipping, showers occurred for 5 consecutive days, and a total of 3.79 inches of rain fell. This rain apparently reduced the period of effectiveness of the dipping by at least 1 week and possibly longer. Total rainfall during the period following the third dipping was 3.05 inches. The treatment appeared to be moderately effective through the third week.

The cattle used in these tests were examined February 11, 1960, for grubs with the results as given in table 2. These data indicate that excellent control of the common cattle grub resulted from dipping three times. Two dippings produced results comparable to those obtained in previous work on 1,185 head sprayed with a 0.5% concentration in which the average count on the sprayed animals was 0.32 grub per head.

Results of analyses of the dip (table 3) show a rather interesting picture. Predip samples, although taken after agitation that appeared to stir the material in the vat thoroughly, were invariably lower than expected. The cattle moving through the vat seemed to do a better job of getting the Co-Ral into suspension than other methods used.

[1] Accepted for publication October 24, 1960. Partial cost of publication of this paper was met by the Chemagro Corporation, Kansas City, Mo.

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Table 3.—Analyses for the concentration of Co-Ral in the vat during dipping tests.

DATE 1959	PER CENT CONCENTRATION		
	Predip	Range	Average
June 18	0.16	0.21–0.23	0.22
July 17	.17	.12– .29	.24
Aug. 20	.12	.19– .21	.20

CONCLUSION.—The data obtained in this study indicated that horn fly control was excellent to moderate up to 3 weeks in spite of considerable rain. Cattle grub control was excellent in the animals dipped three times at the concentration of Co-Ral used, and very good on animals dipped only twice. The analyses for Co-Ral in the dip indicate that the relationship between the removal of the insecticide and the water is nearly linear. Concentration tends to go up with evaporation of the water and down with addition of water through rainfall. The vat material should be measured before and after each dipping and adjustments should be made to maintain the proper concentration. Dilution by rainfall must also be taken into consideration.

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Residues of Phosdrin® on Alfalfa and Its Effectiveness on the Insect Complex[1]

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Most organophosphorus insecticides are short-lived and show less propensity for storage and accumulation in fat and secretion in milk of dairy cattle than chlorinated hydrocarbon insecticides. Hence these compounds are of especial interest to forage entomologists.

Phosdrin® is a highly toxic, systemic organophosphorus insecticide composed of the *cis* and *trans* isomers of 2-carbomethoxy-1-methylvinyl dimethyl phosphate (CMDP). Its toxicity and physiological action is much like that of parathion and TEP (Anonymous 1957).

Information was lacking on the persistence of CMDP, its effectiveness on the insect complex, and on its phytotoxicity to alfalfa. Therefore in 1956 a study of these factors was undertaken at Minetto, New York.

MATERIALS AND METHODS.—A large field of 3-year-old Ontario variegated alfalfa was laid out in a series of 20- by 80-foot plots replicated twice in a completely randomized design. Phosdrin, formulated as a technical emulsifiable concentrate, was applied on July 30, 1956 at dosage levels of 2 or 4 oz. of actual toxicant per acre. This single application was made with a low pressure, low gallonage, "Yellow Devil" sprayer to the 6- to 10-inch alfalfa using 20 gallons of spray per acre at 40 p. s. i. of pressure.

Table 1.—Numbers of various species of forage insects after one day, one week, and two weeks following treatment with Phosdrin at 2 and 4 ounces per acre. Minetto, New York, 1956.

	NO. OF INSECTS[a] IN PHOSDRIN AND CHECK PLOTS AFTER								
	24 Hours			1 Week			2 Weeks		
	2[b]	4[c]	Check[d]	2	4	Check	2	4	Check
Meadow spittlebug	10	15	115	10	30	115	50	70	50
Potato leafhopper	0	0	5	0	0	10	0	10	5
Six-spotted leafhopper	0	0	25	15	10	15	10	25	5
Tarnished plantbug	0	0	10	0	0	5	0	35	5
Pea aphid	0	0	10	0	0	10	15	5	5
Grasshoppers	80	5	325	16	9	305	55	10	130

[a] Per 100 sweeps with an insect collecting net.
[b] 2 ounces per acre of Phosdrin.
[c] 4 ounces per acre of Phosdrin.
[d] Check—Untreated.

The plots were divided arbitrarily in half, and the east portions were sampled for residue analysis at 0, 1, 2, 4 and 7 days following a sampling scheme described by Huddleston (1960). The west portions were sampled at 1-, 7- and 14-day intervals with a 15-inch diameter insect sweep net to determine the effectiveness of the insecticide on the insect complex which consisted of tarnished plant bugs, *Lygus lineolaris* (P. de B.); meadow spittlebug, *Philaenus leucophthalmus* (L.), adults; potato leafhopper, *Empoasca fabae* (Harr.); pea aphid, *Macrosiphum pisi* (Harris); six-spotted leafhopper, *Macrosteles fascifrons* (Stål); and other leafhoppers but largely of red-legged grasshoppers, *Melanoplus femur-rubrum* (DeG.); and two-striped grasshoppers, *Melanoplus bivittatus* (Say).

The alfalfa samples for residue analysis immediately after cutting were composited for each plot, placed in polyethylene bags and frozen until analyzed. Just prior to analysis, the alfalfa was extracted with chloroform and cleaned up using the wax sorption technique. Final analysis was by the enzyme inhibition spectrophotometric method. The method is described in detail in "Determination of Phosdrin Residues in Agricultural Products and Animal Tissues."

In another experiment Phosdrin was applied, in a manner described previously, to Narragansett variety alfalfa in small duplicate plots at 2, 4 and 8 ounces per acre on August 22 to determine the safety of Phosdrin to the plants.

RESULTS AND DISCUSSION.—None of the dosages of Phosdrin used in these experiments caused phytotoxicity to the alfalfa although in an earlier test in early spring Phosdrin at 4 ounces per acre caused a small amount of plant injury on the tender young growth.

Phosdrin was found to be highly toxic to many insects especially at the higher dosage. It was particularly effective against grasshoppers although pea aphids, plant bugs and leafhoppers were readily killed also (table 1).

The residues of Phosdrin were very short-lived and even at 4 ounces per acre were dissipated in 4 days (table 2). Because of its short persistence and high degree of effectiveness it would appear that Phosdrin would be useful in a forage program where treatment might be needed close to harvest. A build-up in pea aphids, a late serious infestation of armyworm (*Pseudaletia unipuncta* (Haw.)), or an influx of grasshoppers just prior to hay harvest are situations where Phosdrin might be useful. Practical considerations, however, such as cost and acute toxicity to warm-blooded animals probably preclude its general use as a forage insecticide.

[1] Accepted for publication September 26, 1960.
[2] The authors are indebted to Northeast Regional Project N.E.36 for their financial support in part of this work and to the Shell Chemical Corporation for assistance.

Table 2.—Residues in p.p.m. (green weight) of Phosdrin on alfalfa at varying intervals between treatment and harvest. Minetto, N. Y. 1956.

Treatment	Actual Toxicant per Acre (oz.)	Interval-Treatment to Sampling (days)	Toxicant Found (p.p.m.)[a]
Phosdrin	2	0	1.8
	2	1	0.49
	2	2	0.07
	2	4	<0.05
	2	7	<0.05
	4	0	2.8
	4	1	0.7
	4	2	0.28
	4	4	0.05
	4	7	0.05
Untreated	—	—	0.02

[a] Analytical results are corrected for apparent toxicant value obtained on the untreated check. Phosdrin was added at 0.05 p.p.m. and 0.10 p.p.m. to untreated alfalfa extract and analyses were performed to check the analytical method. Recoveries were 92% and 88%, respectively.

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An Electrically Heated Retaining Pen for Insect Larvae[1]

J. P. Hollingsworth,[2] C. P. Briggs III,[2] and A. L. Scales[3]

A relatively simple method of restricting certain lepidopterous larvae to a small open area has been needed by entomologists for use in testing the insecticidal effectiveness of various compounds under laboratory conditions. Overhead caging is not always desirable or practicable for this work. Attempts to retain larvae by means of water barriers, chemical repellents, and various structural types of enclosures have proven unsuccessful, particularly for the bollworm (*Heliothis zea* (Boddie)). The work reported herein was undertaken in order to study the possibilities of and requirements for using electricity for retaining larvae in a small area, and to develop equipment and techniques for its application.

Procedure.—The initial work consisted of the use of high a.c. voltage applied to a wire fence around an area in which larvae were present. Although some larvae were repelled, the more persistent ones could escape over the fence. To further test the use of high voltages, a special board containing cells for individual larvae was constructed. The interior wall of each cell was covered by a metal sleeve, which was electrically grounded, and the cell was partially covered with a metal plate connected to a high voltage source. The arrangement was such that the larvae had to contact the grounded metal electrode and high voltage electrode simultaneously in order to escape. Alternating voltage potentials up to several thousand volts were tested and it was found that voltages high enough to prevent larval escape would also electrocute them. It was apparent from these investigations that the use of the high voltage "shock" technique would not be feasible because of the electrocution problem. Work then was directed toward the use of thermal barriers.

Initial work with thermal barriers indicated that heated wires caused extreme discomfort and repelled the larvae. However, on a flat surface some larvae were able to crawl over the heated wires regardless of the temperature levels maintained. It was thought possible that a heated vertical surface would solve this problem. A 2′×2′×4″ metal frame, which had been previously tested as a mechanical barrier, was heated by means of a soil-heating cable taped around the outside of the frame. Work with this device indicated good possibilities for the heated vertical surface. However, this particular arrangement proved unsatisfactory because of thick insulation on the cable and the high rate of heat dissipation by the metal frame.

A wooden frame of the same dimensions as the metal frame then was constructed and approximately 23 feet of 24-gauge nickel-chromium resistance heating wire (Chromel "A" or Nichrome V) was stapled to the inner surface. An adjustable autotransformer was used to provide a variable voltage to the resistance wire for heat control. It was found that three rows of wire spaced three-eighths inch apart gave a heated area of sufficient size to retain the larvae. The lower wire was placed 1½ inches above the bottom of the frame, so that the larvae had to ascend a vertical wall to make contact. The usual reaction was for the larvae to then fall back down into the enclosure. If contact with the lower wire failed to repel the larvae, the second wire, three-eighths inch higher, would then do so. The third, or upper wire, was added as a precautionary measure, although no larvae would pass the middle wire when proper wire temperatures were maintained.

The heating wire finally selected for use is provided with an asbestos covering to prevent possible shock to the larvae and to personnel operating the equipment. Wire temperature requirements were found to be low enough to permit direct mounting of the wire on the wooden frame without danger of igniting or charring the wood. Figure 1 shows frame dimensions and wire placement as used on the experimental unit. Figure 2 is a photograph of the complete unit with accessory equipment.

Operational Characteristics.—Under ambient temperature conditions of approximately 78° F., it was found that approximately 50 volts input to the unit would maintain the wire at the optimum temperature for retaining the larvae within the pen. With this input voltage, the wire temperature is approximately 185° to 190° F. (as measured with a small thermocouple) and the power input is approximately 70 watts. Table 1 shows wire temperature, current, and power consumption for various a.c. input voltages between 25 and 65 volts. Staple temperatures are also shown to emphasize the importance of arranging the staples in such a manner that a continuous exit path for the larvae via the staples is avoided. The proper wire temperature is easily determined by observing the larvae or, after some experience in operation, by touching the wire with the tips of the fingers. As shown in table 1, the current demand is approximately 1.5 amperes at 55 volts so it is therefore possible to operate five units of this type from a single variable voltage transformer having a maximum rating of 7.5 amperes. This voltage should be adequate for maintaining optimum wire temperatures under the ambient temperature conditions ordinarily encountered in environments where work with insects is being conducted. For ambient temperatures in the vicinity of 50° F., an input voltage of approximately 60 volts would be required.

[1] In cooperation with the Texas Agricultural Experiment Station. Accepted for publication November 3, 1960.

[2] Agricultural Engineering Research Division, Agricultural Research Service, United States Department of Agriculture, College Station, Texas.

[3] Entomology Research Division, Agricultural Research Service, United States Department of Agriculture, College Station, Texas.

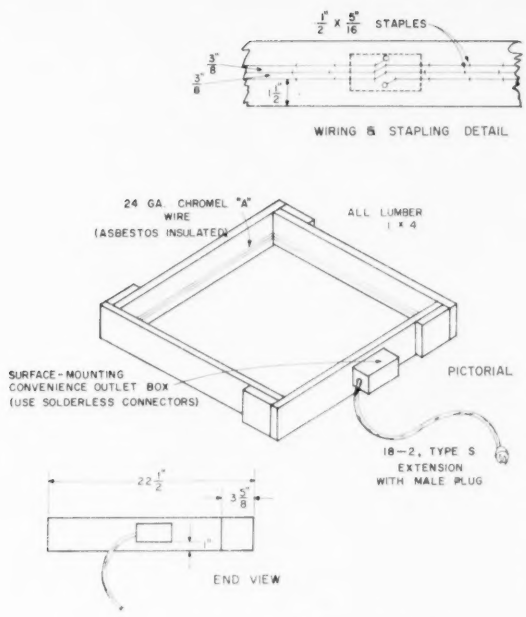

FIG. 1.—Detail drawing of electrically heated insect retaining pen.

This would limit a 7.5 ampere transformer to a capacity of only four retaining pens.

PERFORMANCE.—Five experimental retaining pens of the type shown in figures 1 and 2 were constructed and used in toxicity experiments during 1959. The retaining pens were mounted on a stand as ordinarily used with a standard 2′×2′×2′ toxicity cage.

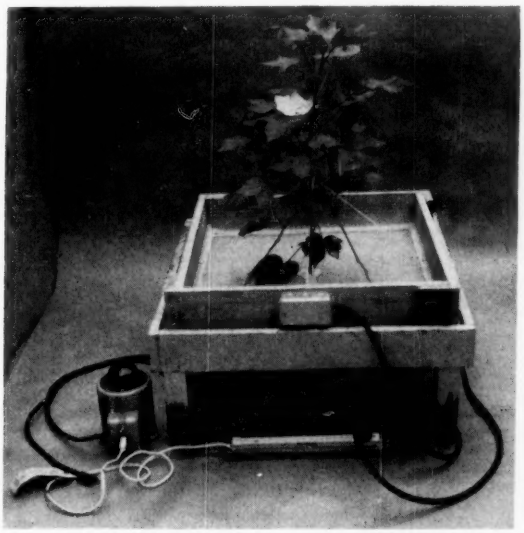

FIG. 2.—Variable voltage transformer, retaining pen, and stand with cotton plant in place as used in toxicity experiments.

Table 1.—Typical operational characteristics of an electric retaining pen for insect larvae. College Station, Texas, 1959.

INPUT VOLTAGE (A.C. VOLTS)	INPUT CURRENT (AMPS)	INPUT POWER (WATTS)	TEMPERATURE (° F.)[a]	
			Wire	Staple
25	0.69	17.1	116	102
30	0.83	24.8	125	108
35	0.97	33.9	138	116
40	1.11	44.4	153	124
45	1.25	56.3	167	133
50	1.38	68.3	187	144
55	1.51	82.1	206	154
60	1.65	98.0	226	165
65	1.79	115.3	244	181

[a] Measured with thermocouple mounted underneath staple next to wooden frame.

For the evaluation tests, individual cotton plants, as shown in figure 2, were treated with various insecticides and then known numbers of second- to fifth-instar larvae of insects such as the cotton bollworm (*Heliothis zea* (Boddie)), cotton leafworm (*Alabama argillacea* (Hbn.)), salt-marsh caterpillar (*Estigmene acrea* (Drury)), and yellow-striped armyworm (*Prodenia ornithogalli* Guen.) were placed on the plant. The nature of these larvae is such that they will occasionally crawl away from the plant unless retained in some manner. The electrically heated retaining pen replaced the screened cage which would ordinarily be used for this purpose. It was found that the electrically heated pen retained all of the above mentioned larvae in a satisfactory manner. This procedure permits an accounting of all larvae used in each test. Elimination of the cage allows free access to the plant for treating and for making the necessary frequent observations.

The Effects of Gamma-Ray Irradiation on Certain Species of Stored-Product Insects[1]

NORMAN M. DENNIS,[2] *Stored-Product Insects Laboratory, Savannah, Georgia*

The use of waste products from nuclear reactors as a source of radiation for killing insects offers a possible new method of stored-product insect control. This paper reports the results of a preliminary study made during 1952 on the effects of gamma-rays from radioactive cobalt on several species of stored-product insects.

PROCEDURE.—The tests were directed toward the determination of the minimum lethal radiation dosages for the adults and immature forms of stored-grain infesting insects. A study of sterility effects produced by sublethal dosages to the confused flour beetle adult, *Tribolium confusum* Duv., was also undertaken.

The 11-Curie Co-60 source used to treat the test insects was loaned to the Entomology Research Division by the Biology Division of Oak Ridge National Laboratory for experiments on

[1] Accepted for publication October 31, 1960.

[2] At the time this work was done, the author was stationed at the Manhattan, Kansas, laboratory of the Stored-Product Insects Branch, Agricultural Marketing Service, U. S. Department of Agriculture.

Radiation performed by R. C. Bushland, Section Head, Insects Affecting Animals Section, Agricultural Research Service, U. S. Department of Agriculture, Kerrville, Texas.

screw-worm (*Callitroga hominivorax* (Cqrl.)) sterilization. The source has been described by Bushland & Hopkins (1953). Insects were irradiated with an aluminum chamber at a dose rate of 4200 ± 10% roentgens per hour. The chamber was 2 inches in diameter and 8 inches long. The chamber was closed during irradiation so there was no air exchange. Exposures were also made at room temperatures which varied considerably. These variables may have been significant during prolonged irradiation.

The test insects were laboratory reared at a temperature of 80° F. and 60% relative humidity. They were packaged and irradiated in ventilated gelatin horse capsules (fig. 1) with various grain or grain products as culture food. The capsules were mailed to Dr. R. C. Bushland for irradiation at the Entomology Research Division's Kerrville, Texas, laboratory and returned by mail to Manhattan, Kansas, for observation. A duplicate set of test insects accompanied each shipment and was returned untreated to Manhattan. These served as untreated controls. All of the test insects, upon return to the Manhattan laboratory, were removed from the horse capsules, placed in sterile culture media and incubated under controlled rearing conditions for the observation periods. The results of the various exposures are given in table 1.

RESULTS.—The mortalities obtained in the various exposure periods are given in table 1. An exposure to 100,800 r killed all of the confused flour beetle adults and larvae, saw-toothed grain beetle adults, lesser grain borer adults, granary weevil adults, rice weevil adults, Indian-meal moth larvae, and the almond

FIG. 1.—The gelatin horse capsules used to confine the test insects during shipment in the mail and in the irradiation chamber at Kerrville, Texas.

Table 1.—The effects of gamma-ray irradiation of species of stored-product insects exposed to different amounts of radiation.

Hours of Exposure	Amount of Radiation (roentgens)	No. Insects Exposed	Insect Tested	Stage	Culture Media	Per Cent Mortality After 6 Days	Per Cent Mortality After 2 Months
2	8,400	25	Confused flour beetle	Adult	Wheat flour	0	[a]
4	16,800	25	Confused flour beetle	Adult	Wheat flour	0	[a]
6	25,200	25	Confused flour beetle	Adult	Wheat flour	0	[a]
12	50,400	25	Confused flour beetle	Adult	Wheat flour	28	28
24	100,800	25	Confused flour beetle	Adult	Wheat flour	44	96
24	100,800	25	Confused flour beetle	Larval	Wheat flour	8	100
24	100,800	25	Saw-toothed grain beetle (*Oryzaephilus surinamensis* (L.))	Adult	Rolled oats	0	100
24	100,800	25	Lesser grain borer (*Rhyzopertha dominica* (F.))	Adult	Wheat	28	100
24	100,800	25	Granary weevil (*Sitophilus granarius* (L.))	Adult	Wheat	44	100
24	100,800	25	Rice weevil (*Sitophilus oryzae* (L.))	Adult	Wheat	96	100
24	100,800	10	Indian-meal moth (*Plodia interpunctella* (Hbn.))	Larval	Dog food mix	0	100[b]
24	100,800	10	Almond moth (*Ephestia cautella* (Wlk.))	Larval	Flour	0	100[b]
36	151,200	25	Saw-toothed grain beetle	Adult	Rolled Oats	100	100
36	151,200	25	Confused flour beetle	Adult	Flour	100	100
36	151,200	25	Confused flour beetle	Adult	Flour	100	100
36	151,200	25	Confused flour beetle	Larval	Flour	100	100
36	151,200	25	Indian-meal moth	Larval	Dog food mix	100	100
48	201,600	25	Confused flour beetle	Larval	Flour	100	100
No Treatment—control:							
		25	Confused flour beetle	Adult	Flour	36	44
		25	Confused flour beetle	Larval	Flour	4	12
		25	Saw-toothed grain beetle	Adult	Rolled Oats	4	8
		25	Lesser grain borer	Adult	Wheat	4	16
		25	Granary weevil	Adult	Wheat	8	32
		25	Rice weevil	Adult	Wheat	40	48
		10	Indian-meal moth	Larval	Dog food mix	0	20
		10	Almond moth	Larval	Flour	0	10

[a] Not observed.
[b] Dead adults.

Table 2.—Reproduction of the test insects not exposed to gamma-ray radiation.[a]

Original No. of Test Insects	Species	Stage	No. Insects After Exposure					
			6 Days		2 Months			
					Larvae	Pupae	Adults	
			Live	Dead	Live	Live	Live	Dead
25	Confused flour beetle	Adult	16	9	24	6	18	11
25	Confused flour beetle	Larval	20	1	7	1	27	3
25	Saw-toothed grain beetle	Adult	24	1	31	17	29	2
25	Lesser grain borer	Adult	24	1	[b]	[b]	35	4
25	Granary weevil	Adult	23	2	[b]	[b]	45	8
25	Rice weevil	Adult	15	10	[b]	[b]	62	12
10	Indian-meal moth	Larval	10	0	22	7	11	2
10	Almond moth	Larval	10	0	34	4	9	1

[a] No reproduction occurred in test insects exposed to 8,400 r for two hours, the shortest exposure and least amount of radiation used in these tests.
[b] Internal forms were not observed.

moth larvae, within 2 months. An exposure to 151,200 r killed the saw-toothed grain beetle adults, confused flour beetle adults and larvae, and Indian-meal moth larvae within 6 days.

Confused flour beetles exposed to 8,400 r did not reproduce during a 2-month period after exposure, but the test insects in the control reproduced normally, table 2.

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Population Fluctuations of *Lydella grisescens*, a Parasite of the European Corn Borer[1]

J. L. Jarvis and G. T. York, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*[2]

Baker *et al.* (1949) and Thompson & Thompson (1923) have discussed in detail the biology of *Lydella grisescens* R.D., an imported parasite of the European corn borer (*Pyrausta nubilalis* (Hbn.)). However, no account was given of seasonal population fluctuations of *Lydella*. York *et al.* (1955) found that in Iowa *Lydella* adults emerge too early in the spring to parasitize the corn borer and as a result produce one generation on the stalk borer (*Papaipema nebris* (Guen.)), and at least two additional generations on the corn borer. Blickenstaff *et al.* (1953), in a report on parasites of the corn borer in Iowa, summarized the releases and recoveries of exotic parasites. *Lydella* was never actually released in Boone County but was first recovered there in 1950.

Extensive collections of corn borer larvae were made in Boone County, Iowa, from 1950 through 1959. These larvae were processed at the Ankeny, Iowa laboratory to determine the percentage parasitized by *Lydella*. The results are shown in table 1, and are accompanied by corn borer populations recorded for the same periods. In several instances there were no collections made for parasite analysis.

After the initial establishment of *Lydella* in Boone County in 1950, the population remained very low until the fall of 1953. Since that time, the percentage of borers parasitized by *Lydella* has fluctuated somewhat. A peak occurred in the fall of 1954 and extended into 1955. However, by 1956 the population was again low. The percentage of parasitization was much larger in 1957, and reached another peak in the fall of 1958, but again declined in 1959. The percentage of first-generation corn borer larvae parasitized by *Lydella* has always been small.

Table 1.—European corn borer populations and per cent parasitized by *Lydella grisescens*, Boone County, Iowa, 1950–1959.

Year	Season	Number of Borers		Per Cent of Borers Parasitized
		Processed	Per 100 Plants	
1950	Spring	3,370	—	0
	Summer	5,525	146	.2
	Fall	1,983	110	.1
1951	Spring	1,649	—	.1
	Summer	148	22	0
	Fall	1,127	78	.5
1952	Spring	—	—	—
	Summer	—	5	—
	Fall	812	122	.5
1953	Spring	—	—	—
	Summer	114	27	.9
	Fall	415	271	4.3
1954	Spring	234	—	5.1
	Summer	712	223	.4
	Fall	823	560	15.4
1955	Spring	107	—	19.6
	Summer	140	44	.7
	Fall	145	153	11.0
1956	Spring	83	—	2.4
	Summer	137	43	0
	Fall	187	178	2.7
1957	Spring	307	—	2.3
	Summer	—	57	—
	Fall	209	207	11.5
1958	Spring	381	—	3.4
	Summer	—	88	—
	Fall	220	128	24.1
1959	Spring	72	—	15.3
	Summer	—	7	—
	Fall	30	10	10.0

[1] Accepted for publication November 4, 1960.
[2] In cooperation with Iowa Agricultural Experiment Station.

There is no apparent relationship between population fluctuations of *Lydella* and those of the corn borer. The low populations of *Lydella* in Boone County prior to 1954 were because of the failure of the parasite to become abundant following its initial establishment. However, population fluctuations of Lydella since 1953 cannot be explained on the basis of corn borer populations. In view of the findings of York *et al.* (1955), it is possible that they may be partially explained by the scarcity or abundance of the stalk borer. No data are available to support this assumption.

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NEW PRICE SCHEDULES

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A price schedule for reprints of papers from the periodicals of the Entomological Society of America was adopted by the Governing Board at the 1955 meeting in Cincinnati. The schedule was printed on page 35 of the December 1955 BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA. This schedule has remained in effect until this writing in spite of the fact that the cost of the reprints to the Society was increased in 1957 and again in 1960. The Governing Board at the 1960 meeting in Atlantic City carefully considered the matter and voted to increase our prices. We hope it will be possible to maintain this new schedule as well as we did the previous one.

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BOOK REVIEWS

FOREST and SHADE TREE ENTOMOLOGY, by ROGER F. ANDERSON.

Published by John Wiley & Sons, Inc., New York 1960. vii+428 pages, Price $8.50.

Anderson has authored a book on forest insects which is both a textbook and a manual of forest, shade tree, and wood products entomology. It is a very welcome and valuable contribution to forest entomology literature that is designed to help forestry and entomology students obtain an understanding of insects that attack forests and forest products and to acquaint them with the methods used in combating them.

This book has been developed as an aid to teachers and students of forest entomology and is based on the author's many years of experience in fields of research and teaching. It is divided into two sections: the first dealing with basic aspects of entomology and the second, treating of specific forest insects. Section I which comprises about one-fourth of the text discusses structure, physiology and development of insects; their classification and ecology. Chapters in this section are also devoted to forest insect surveys and control, and to insecticides and methods of their application.

Section II, which comprises three-fourths of the text, treats individually the more important insect pests of forests, shade trees, and wood products. Chapters in this section are devoted to a discussion of defoliating insects, bark beetles, and wood boring and sap sucking insects; to those that damage buds, twigs and seedlings, and roots; and to cone and seed destroying insects.

The author has made liberal and excellent use of numbered tables or keys designed to aid in the identification of insect orders, genera, and species. These keys often combine information on taxonomy, geography, host, nature of injury, or other useful field characteristics. They should prove exceedingly helpful to users of the book in identifying many of the forest insect pests with which they may be dealing.

The text is amply illustrated throughout with many of the plates portraying the life stages of selected examples of economic pests or of the nature of injury which they cause.

As a guide to the reader, liberal and commendable use has been made of headings, subheadings and sideheadings. The text is clear and forthright and the author's style makes it a very readable book. Errors are few and are not worthy of comment here. The art work is good and reproduction of both the illustrations and the text is excellent.

This book fills a long-felt need in the teaching of forest entomology and will be helpful to teacher and student alike. As a manual, it should also prove useful to entomologists, foresters, extension workers and other individuals concerned with forest insect problems. In short, it is a guide to the scientific understanding, identification and control of forest insects.

JAMES A. BEAL, Director
Division of Forest Insect Research
Forest Service, USDA

THE TICKS, OR IXODIDES, OF THE U.S.S.R.—A REVIEW OF THE LITERATURE, by GEORGE ANASTOS. Public Health Service Publ. No. 548, U. S. Dept. of Health, Education and Welfare. vi+397 pp. 1957. (Processed).

The author has performed an admirable task of reviewing the extensive Russian literature on the ticks of that country, and assembling the information in logical and useful form.

It is pointed out that more than 1,000 references were available and about one-half of these were seen either in the original or abstract form.

In this work 72 species or varieties are treated. These are made up of 3 species of *Argas*, 8 species of *Ornithodoros*, 1 *Boophilus*, 10 *Dermacentor*, 12 *Haemaphysalis*, 4 *Rhipicephalus*, 7 *Hyalomma*, and 21 Ixodes. In instances where a species is not endemic to Russia some information is included on its occurrence in other countries.

Under each species information is given on the synonymy, geographical distribution, host distribution, hosts of various stages, seasonal activity, habitat, life cycle, habits, control measures, parasites and relation to disease.

The author has followed, rather closely, the important work of B. I. Pomerantzev (1950) in listing most of the species occurring in the U.S.S.R., but has made some changes to bring the work in line with the concept of tick nomenclature held by American and other European taxonomists.

The names of the hosts were checked by specialists in the U. S. National Museum.

This summary of information on ticks published by Russian workers will be of inestimable value to students of these important pests and disease carriers, in various parts of the world where Russian literature is not readily available or easily translated.

FRED C. BISHOPP

BEEKEEPING, by JOHN E. ECKERT and FRANK R. SHAW. The Macmillan Company, 1960. 536 pages, $12.50.

This book is offered as a successor to "Beekeeping" by E. F. Phillips, which was published in 1915. Dr. Phillips was a man of great stature in the field of apiculture as a teacher, a researcher, and a writer. His book was written with perfect clarity and was not only the most factual in its time but also contained much sound philosophy of value to the student in the academic sense as well as the practical beekeeper. He had the happy faculty of holding the interest and at the same time stimulating the imagination of the reader. The present book is aptly described as a successor to Dr. Phillips' book, as it is in no sense a revision. It is a completely new book although in a few instances it follows the form and adopts some of the thoughts of the original.

The book contains 24 chapters, each dealing with a different phase of beekeeping. The authors are men of wide experience in the field and have done a very commendable job of picking out the most essential factual information and bringing it up to date. This is not an easy task at the present time when we realize that books have been written on the subject matter contained in some of the chapters and a book could be written on any one of the 24 chapters. Comprehensive books on beekeeping are generally read by many beekeepers and may also be used as college texts. This imposes a dual responsibility on the authors. In general "Beekeeping" does a noteworthy job in this respect, although there is some unevenness. For instance, the section on embryology, with the free use of technical terms, would dishearten the average beekeeper while the great amount of how-to-do-it detail, which is a little hard to follow in spots, would be top heavy for the average student.

Chapters on honey and queen rearing are particularly well presented. The chapter on pollination summarizes the information in this important field very effectively. There is somewhat of a tendency to take up the banner for the honey bees versus the wild bees for pollination. The overall value of all bees in perpetuating many thousands of angiosperm plants that cover the earth's surface is so great that there is plenty of room for credit for both groups.

Any comments should not be construed as detracting seri-

ously from a book which the reviewer would highly recommend to all who are interested in bees and beekeeping.

E. C. MARTIN
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FAUNA OF U.S.S.R. ARACHNIDA VOL. IV NO. 2. IXODID TICKS (IXODIDAE) by B. I. POMERANTZEV. Zoological Institute of the Academy of Science No. 41, 218 pp., 638 figs. Moscow. 1950. Translation by ALENA ELBL. Edited by GEORGE ANASTOS. Amer. Inst. of Biol. Sciences, Washington, D. C. 199 pp. 638 figs. 1959. Price $10.00.

This outstanding contribution to our knowledge of the hard-backed ticks by B. I. Pomerantzev was published about 10 years after his death. The text dealing with each of the several genera was prepared separately from one to several years prior to his death. The material was assembled for this publication by Academician E. N. Pavlovki and Pomerantzev's wife, G. V. Serdukova. The only material changes in Pomerantzev's original text was in the genus *Rhipicephalus*, written in 1935, which was brought to conform with his more recent views.

The publication deals with 17 species of *Ixodes*, 11 species of *Haemaphysalis*, 1 species of *Boophilus*, 8 speices of *Dermacentor*, 7 species of *Rhipicephalus* and 8 species and 5 varieties of *Hyalomma*. Although these are primarily Russian forms, many of them are distributed much beyond that far-flung Republic, and a few, such as *Ixodes angustus*, *I. putus*, and *Rhipicephalus sanguineus* occur in the U.S.A.

In a brief introduction the importance of ticks as disease vectors is emphasized and it is asserted that "more than 50% of all scientific publications dealing with Ixodoidea for the past twenty years belong to the Soviet Union." Following this is a well-prepared comparative morphological sketch of the ixodid ticks and a list of 68 "major references."

A key to the genera is presented as well as keys to the males and females (where known) of each species. Brief descriptions of the sexes are given, but no descriptions or keys to immature stages are included.

It is noted that the author considers *Boophilus calcaratus* Birula a distinct species whereas many current workers consider it a synonym of *B. annulatus*, our American species, the carrier of Texas or splenetic fever of cattle.

The hundreds of line drawings bring out the principal taxonomic characters very well, but the two dozen photographs, mostly of habitats, failed to reproduce well in this translation.

An interesting and desirable feature of this work is an index to the species given in parallel columns with the text pages in the Russian book and in the translation.

The translator, editor and publishers of this translation are to be complimented on making this important Russian work more readily available to students of ticks in America and other parts of the world.

FRED C. BISHOPP

INDEX XVII

INDEX TO THE LITERATURE OF AMERICAN ECONOMIC ENTOMOLOGY

Number XVII of this series, which was published in 1960, covers the literature for 1958. The book contains 501 pages. Indexes I through XVI covering the literature from 1905 through 1957 are also available.

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OBITUARY

Herbert Dwight Smith
1894-1959

With the passing of Herbert Dwight Smith, who met an untimely death, presumably by accident, in Mexico City on January 21, 1959, at age 64, Entomology and Herbert's family and numerous friends suffered a severe blow.

Herbert, or Herb as he was known to most of his friends and associates, was born in West Haven, Conn., June 29, 1894, the youngest of four children. His mother died when he was 13, and his further care and education devolved upon his father and brother Leon and his two sisters, Edith and Ruth. After graduating from high school in Poughkeepsie, N. Y., where the family lived for some years, he went to the University of Massachusetts (then Massachusetts Agricultural College) at Amherst, Mass., where he received his B.S. in Entomology in 1917. He joined the Navy in 1917, where he served for 2 years as a wireless operator on a sub-chaser, and continued for some time later in the same capacity on commercial boats. In 1920 he joined the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, and was stationed at the Carlisle, Pa., laboratory where he did some very fine investigations and published some excellent papers on the Hessian fly and wheat stem sawfly and their parasites and other insect pests of cereal and forage crops.

In 1928 Herb was transferred to foreign work and spent the next 11 years with the European Parasite Laboratory at Hyeres and St-Cloud, France, working principally on the European corn borer and its parasites and traveling over western Europe and North Africa. He conducted biological control studies at Moorestown and Hoboken, N. J., from 1939–44. From 1944–46, he was stationed in Greece as agricultural officer in the United Nations Relief and Rehabilitation Administration. He then returned to the Bureau of Entomology and Plant Quarantine and served as plant quarantine inspector at the port of New York from 1946–47 after which he conducted explorations in Cuba, India, Malaya, Sumatra, and the Philippines for parasites of the citrus blackfly.

Herb spent the last several years of his life in Mexico, working on biological control of the citrus blackfly, first as a member of the Entomology Research Division and later as a member of the Plant Pest Control Division, Agricultural Research Service. His epochal accomplishment of discovery, handling, importation, colonization and distribution of parasites of the citrus blackfly in Mexico was the crowning entomological achievement of his career. For this work he was honored posthumously at the annual "Dia del Insecto" program of the Mexican Entomological Society and the Department of Parasitology of the National School of Agriculture, held August 17, 1959 at Chapingo, Mexico.

Herb was a modest, gay and friendly man, appreciated and esteemed by all who came in contact with him. He was sufficiently adaptable in languages to acquire rapidly a necessary working knowledge of Italian and modern Greek, and became fluent in French and Spanish. He played tennis and golf and bowled, and while in Europe did considerable mountain climbing and trail-hiking in the Maritime Alps, Prealpi Bergamaschi, and Dolomites of the Alto Adige.

Herb married Miss Catherine Wilson of Glasgow, Scotland, in 1922, by whom he had two daughters, Janet (Mrs. Jean-Jacques Rosier of La Celle St-Cloud, France), and Patricia (Mrs. Frank Stevens of Rutherford, N. J.). Catherine died at Hyeres, France, in 1934. Herb married Miss Jane Wilson of Glasgow in 1935, by whom he had one daughter (Miss Lucienne Smith of New York and Rutherford, N. J.). He is survived by Jane who lives in Rutherford, N. J., his three daughters, and one brother Leon, who is retired, of Shelby, Ohio.

Herb was a member of the Entomological Society of America, and during his residence in France was a member of the Société Entomologique de France and the Club Alpin de France. He was the author of a number of publications dealing with the natural enemies of grain, forage, and citrus insects.

The following tribute to Herb taken from his nomination for the U. S. Department of Agriculture's Superior Service Award, which he received in 1955, is indicative of the character, personality and devotion of Herb Smith:

"In the biological control of the citrus blackfly, Mr. Smith accepted the problems as personal challenges, and the manner in which he met them is representative of the highest type of scientific initiative and devotion to duty. He ignored personal convenience and comfort and frequently worked 'around the clock' in his efforts to supervise the program or attend pressing operational matters in various parts of Mexico. Due largely to his personal efforts, suppression of the citrus blackfly by biologic means throughout the greater part of Mexico is an accomplished fact months or years in advance of the normally expected time.

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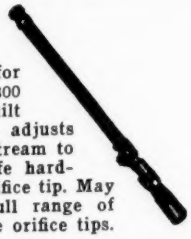

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VOLUME 1, NUMBER 2, pp. 33-68 appeared February 17, 1960 and contains a "Phylogenetic-Systematic Study of Larval Oedemeridae (Coleoptera)" by Jerome G. Rozen, Jr.

VOLUME 1, NUMBER 3, pp. 69-171 appeared July 27, 1960 and contains the following: "Swarming and Mating in Mosquitoes," by Erik Tetens Nielsen and James S. Haeger. "A Revision of the Genus *Chlaenius* Bonelli (Coleoptera, Carabidae) in North America," by Ross T. Bell.

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